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PHYSIOLOGICAL RESPONSE OF POTATO TO PHOSPHORUS NUTRITION AND VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

by

David Albert James McArthur

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
Doctor of Philosophy
IN
Plant Physiology

Department of Plant Science

EDMONTON, ALBERTA
Fall 1992

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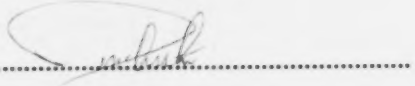

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N. R. Knowles, Supervisor

G. J. Taylor

A. Horak

W. H. Vanden Born

A. Flanagan for Steven J. Crafts-Brandner
(External Examiner)

Date: Oct 5, 1992

DEDICATION

To Christine, for her love, strength, and patience

ABSTRACT

Growth, development, and physiology of potato (*Solanum tuberosum* L.) plants in response to P nutrition and during interactions with four species of vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus* spp.) were investigated. Leaf area development and plant growth were significantly decreased by P deficiency. The extent to which tuber growth was limited by P deficiency depended upon the timing of tuber initiation. P-deficient plants had enhanced levels of non reducing sugars and nitrate, possibly as a result of low rates of root respiration and leaf nitrate reductase activity. The accumulation of mineral nutrients was much less for low-P plants than for high-P plants, indicating a restricted capacity for nutrient uptake. The specific activities of root acid phosphatases and vanadate-sensitive microsomal ATPases were enhanced in P-deficient plants. The establishment of a VAM symbiosis by low-P plants significantly contributed to plant P acquisition, and a greater level of root infection for P-stressed plants indicated enhanced plant compatibility to VAM fungi.

The protein content and microsomal ATPase activity of roots on a fresh weight basis were significantly increased by VAM infection. In contrast, the capacity of excised roots to produce ethylene was markedly decreased by VAM infection. Furthermore, leachate from VAM roots contained a potent water-soluble inhibitor of ethylene generation. As the specific activities of extracellular peroxidase and polyphenol oxidase were unaffected by VAM infection, these results characterize the low resistance response of P-deficient plants to VAM infection. High-P plants showed less of a growth response to VAM infection and had a low level of root infection, indicating high-P plants as less compatible with VAM fungi. In support of this, both ethylene production and extracellular peroxidase activity of roots increased linearly with abiotic P supply, thus indicating a greater potential for resistance to VAM infection.

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List of Abbreviations

ACC	1-amino cyclopropane-1-carboxylic acid
ACC_{ox}	ACC oxidase
ANOVA	analysis of variance
Apase	acid phosphatase
ATPase	adenosine triphosphatase
DAP	days after planting
DOPA	3,4-dihydroxyphenylalanine
FeEDDHA	ferric ethylenediamine di(*o*-hydroxyphenyl) acetic acid
f.wt.	fresh weight
LAR	leaf area:plant dry weight ratio
L_W/W	leaf:plant dry weight ratio
MES	(2-[N-morpholino]ethanesulfonic acid)
MTT	3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-2H-tetrazolium bromide
NM	non-mycorrhizal
*p*NPP	*para*-nitrophenol phosphate
NRS/RS	non-reducing sugars:reducing sugars dry weight ratio
PAGE	polyacrylamide gel electrophoresis
PAL	phenylalanine ammonia-lyase
POX	peroxidase
PPO	polyphenol oxidase
PSI	phosphate starvation induced
RGR	relative growth rate
RSAR	relative specific absorption rate
R_W/W	root:plant dry weight ratio
SAM	S-adenosylmethionine
SAR	specific absorption rate
SDS	sodium docecyl sulfate
SLA	specific leaf area
S/R	shoot:root dry weight ratio
S_W/W	shoot:plant dry weight ratio
Tris	tri(hydroxymethyl)methylamine
T_W/W	tuber:plant dry weight ratio
ULR	unit leaf rate
VAM	vesicular-arbuscular mycorrhizal
wt	weight

Chapter I

Background and Objectives

Soil Phosphorus

Phosphorus is relatively abundant in most soils; however, its availability for absorption by plants is often very limited. Plants derive most of their P from inorganic phosphate in the soil solution, but this fraction constitutes only a small proportion (<1%) of total soil-P (Bolan, 1991). This results from the fact that, in soils with a pH less than 6, phosphate readily combines with ions of iron, aluminum, and manganese and with oxides of iron, aluminum or magnesium to form stable precipitates. Moreover, phosphate is readily adsorbed onto silicate minerals in soil at a pH between 5.5 and 7.0. In soils with a pH greater than 7, P exists as insoluble calcium phosphates. As a result of its reactivity, phosphate tends to be immobile, and to move only slowly by mass flow. Greater movement of phosphate in soils is possible by diffusion, but factors such as low soil temperatures and low soil moisture further diminish phosphate mobility. As a result, localized areas of P deficiency can readily occur around plant roots. The addition of soluble P fertilizers initially raises the solution-phase concentration of soil phosphate; however, 'fixation' rapidly occurs by the processes of inorganic sorption, precipitation, and organic immobilization. Although diminishing with time, these processes continue to significantly decrease the concentration of phosphate in the solution-phase and hence decrease the availability of fertilizer-P to plants.

Plant Acquisition of Phosphorus

Plant species differ widely in their ability to acquire P and in the amount of P needed to reach their maximum yield (Fohse et al., 1988; Koide, 1991; Marschner, 1986). Moreover, differences between species in terms of their exogenous-P requirements and their ability to maintain P uptake and growth, when available P is less than optimum, can be related to their relative allocation of dry matter to root growth, root morphology and the net influx capacity of roots. In this regard, the ability of roots to quickly move out of zones of P-deficiency and to develop a large absorptive surface area appears especially important (Barber and Silberbush, 1984; Clarkson, 1985). Root hairs, for example, may extend out only a millimetre from the root surface; however, it has been estimated that only four milligrams of roots hairs have an equivalent length of 1600 milligrams of roots, assuming an average root diameter of 400 μm and an equal specific gravity (Bowen et al., 1975). Differences in P uptake

efficiency between plant species have been shown to be at least partially related to differences in the amount of root hair formation on roots (Itoh and Barber, 1983).

Under conditions in which available P is severely limiting to growth, roots show a number of inducible adaptations that appear to improve P acquisition. In general, these result in more rapid solubilization of soil P, a greater root affinity for available P and hence, greater P uptake (Clarkson, 1985; Cogliatti and Clarkson, 1983; Goldstein et al., 1988). For example, reducing and chelating activity by root exudates, and proton extrusion by a H^+-pump at the plasma membrane have been shown to solubilize P from calcium phosphates and various phosphate-containing colloids (Gardner et al., 1982; Hedley et al., 1983; Romheld and Kramer, 1983). Also, acid phosphatases bound to the surface of, or in the periplasmic spaces of, plant roots show some capacity for P recovery from organic phosphates (Clarkson, 1985; Hedley et al., 1983). Some root phosphatases may further ameliorate the effects of P-deficiency stress in plants by minimizing the cellular requirements for adenylates and phosphate, both pools of which are diminished by inadequate P (Duff et al., 1991). Although species differ in their ability to utilize such physiological adaptations for growing in P-deficient soils, it is likely that most species are capable of their expression to some degree (Clarkson, 1985; Duff et al., 1991; Goldstein et al., 1988).

Vesicular-Arbuscular Mycorrhizal (VAM) Fungi

One of the most widely recognized adaptations by plants to improve P acquisition requires a partner, a mycorrhizal fungus. Such fungi involve compatible plants in a mutualistic symbiosis and form mycorrhizae (fungus-roots). Mycorrhizal fungi have been found to be almost ubiquitous in soils and there is some diversity in the species involved and in the types of plant-fungal associations that are observed (Kendrick, 1985). In agronomically important plants, however, the most widely observed are the vesicular-arbuscular mycorrhizal (VAM) fungi (Abbott and Robson, 1982; Powell and Bagyaraj, 1984). VAM fungi are obligate biotrophs, as demonstrated by the bi-directional exchange of ^{32}P and ^{14}C-compounds between symbiont and host, and also by their inability to grow in axenic culture (Cox et al., 1976; Hepper, 1984). Once a mycorrhizal fungus has infected a compatible-host root, the fungus produces an extensive network of external hyphae that contributes significantly to the root absorptive capacity for P, although sometimes also for other poorly mobile mineral nutrients such as zinc and copper (Smith and Gianinazzi-Pearson, 1988). Studies show that, although VAM fungi and plants have access to the same pools of P in soil (Bolan, 1991), P uptake can be as much as four-fold higher for VAM roots than for comparable

non-infected roots (Bowen et al., 1975). Measurements on the extent of total hyphal length adding to the absorbing surface of the root range from 0.8 to 14.2 metres of hyphae per centimetre of infected portion of root (Smith and Gianinazzi-Pearson, 1988).

VAM fungi are a small group of asexually reproducing, symbiotic fungi that have been classified into the subdivision Zygomycotina, order Glomales (Morton and Benny, 1990). One of the most commonly observed and studied genera of these or of any mycorrhizal fungi, is the genus *Glomus*, which is further classified into species by the characteristics of sporocarps, if present, and by spore morphology and reaction to various reagents (Berch, 1988). As is typical for VAM fungi, species of *Glomus* form compatible associations with species of most angiosperm families, many of which are agonomically important. Microphotographs of fossil remains of root-like structures for *Asteroxylon* and *Rhyia* plant species, dating to a period about 370 million years ago show within them the presence of fungus-like structures bearing a strong resemblance to those typical of *Glomus*-infected roots (Kidston and Lang, 1921 cited by Nicholson, 1975). Hence, it has been suggested that this endophyte-plant relationship and the VAM symbioses in general are ancient associations, likely of mutual benefit, that have co-evolved with plants (Nicholson, 1975). The limited availability of P for plant growth then, as it is now, could have been the basis for selection and perpetuation of these fungus-root (mycorrhizal) associations.

Characteristics of VAM (*Glomus*) Roots

Although the temporal and physiological patterns for root infection and establishment of the symbiotic condition by all VAM genera have not been fully characterized (Koide and Schreiner, 1992; Smith and Gianinazzi-Pearson, 1988), light and electron microscopy of plants infected by *Glomus* species have provided a framework for the understanding of physical and molecular events leading to the VAM condition. The pattern of fungal growth intraradically may differ somewhat depending upon *Glomus* and plant species, and plant nutritional status (Braunberger et al., 1991), however, the development of root morphology has been sufficiently described to characterize VAM roots in a general way.

Initially, a hypha from a soilborne chlamydospore contacts a compatible zone of root, and initiates an appressorium. After penetration, the hypha grows longitudinally in the outer cortical cells of the root, branching both inter- and intracellularly. The hyphal wall is closely appressed to the plant cell wall, as is typical of other symbiotic associations such as lichens and ectomycorrhizae (Carling and Brown, 1982; Harley and

Smith, 1983; Holley and Peterson, 1979). Intracellular hyphae often coil within a cell, but are separated from the cell protoplast by a matrix of material similar to that of plant primary cell wall. The inter- or intracellular hyphae can expand to form vesicles, which resemble chlamydospores in size and constituents. An intracellular vesicle may fill the cell lumen so that the matrix material and cell cytoplasm are reduced to a compressed layer around the fungal wall. Although variable, the appearance of external hyphae from intraradical growth occurs after some colonization of the root, and often coincides with vesicle formation. These external hyphae can re-infect the same or other plant roots, or ramify out into the soil (Rhodes and Gerdemann, 1975). Intraradical hyphae may also differentiate to form arbuscules. During this process, a lateral branch of an intra- or intercellular hypha grows into the cell lumen and the protoplast, to accomodate its displacement, invaginates around the hypha and deposits a granular cell-wall material around it. The hypha branches repeatedly in a dichotomous manner to produce a highly branched haustorium-like structure known as the arbuscule. With the process of branching, the wall of an arbuscular-hypha thins, and loses much of its chitin component. Soon afterwards, the hypha terminates elongation altogether. Around the terminal hyphal branches, the matrix material deposited by the protoplast thins to a loosely fibrillar substance. This thin interfacial zone between symbiont and host has been histochemically characterized as consisting mainly of loosely-organized primary plant-cell-wall material (Bonfante-Fasolo et al., 1981; Scannerini and Bonfante-Fasolo, 1979).

It was estimated by Cox and Tinker (1976), using electron micrographs, that a protoplast increases the surface area of its plasma membrane three-fold and its tonoplast two-fold to surround an arbuscule. During arbuscule and cell interactions, the cell nucleus and nucleolus are greatly enlarged, and often are found in close association with the advancing arbuscule (Carling and Brown, 1982). Such nuclear enlargement appears to involve mechanisms other than DNA endoreduplication (Blair et al., 1988). Moreover, the amount of cell cytoplasm may increase 23-fold, with a proportional increase in cell organelles. The large central vacuole, which formly occupied most of the cell lumen, fragments into many minute vacuoles (Scannerini and Bonfant-Fasolo, 1983). Finally, arbuscule deterioration begins days after its completion, lasting as long as 14 days. During the process of arbuscule deterioration, the fungal cytoplasm retreats and seals off the arbuscule, while the plant cell gradually returns to a metabolic state typical of a non-infected cortical cell.

Observations on the influence of the invading arbuscule on cell metabolism have generated comparisons of this type of cell to the transfer cells that exist in some plant

species and in root cells of plants that show the iron-stress response (Clarkson, 1985; Romheld and Marschner, 1981). The arbuscule, which is at an elevated level of metabolic activity and in close association with the cell, often is considered to be the conduit for transfer of P to the cell, and the channel for carbohydrates from the cell for fungal lipid biosynthesis (Gianinazzi-Pearson and Gianinazzi, 1986; Scannerini and Bonfante-Fasolo, 1983; Smith and Smith, 1986)

Symbiont - Host Nutrient Exchange and Compatibility

Research interest in the area of nutrient exchange has been especially high as elucidation of the mechanism by which exchange occurs may also provide insights into the basis for the broad host-specificity of VAM fungi, and host - parasite interactions in general. In early studies on the influence of P nutrition and photosynthesis on VAM fungal growth, much circumstantial evidence accumulated indicating a direct relationship between the rate of root exudation of carbohydrates and root infection. Leakage of carbohydrates was attributed to an impairment of plasma membrane integrity by P deficiency (Graham et al., 1981). Thus, fungal growth was supported by P-deficient plants, but not by P-sufficient plants. It was also proposed that infected roots obtained P from VAM fungi during the deterioration (digestion) of arbuscules, which were known to contain polyphosphates (Bowen *et al.*, 1975; Callow *et al.*, 1978; Cox *et al.*, 1980). This model of passive exchange of nutrients, however, cannot account for the rate of flux of either carbon or P between symbiont and host (Schwab et al., 1991). In the only study of its kind, Capaccio and Callow (1982) succeeded in isolating apparently intact intraradical hyphae and arbuscules from mycorrhizal roots by cell wall digestion. From such fungal structures, the activity of polyphosphatases was demonstrated to be high in intraradical hyphae compared to external hyphae, while the opposite was true for the activity of polyphosphate kinase. Since polyphosphates stream from external hyphae to arbuscules (Cooper and Tinker, 1981), these findings have provided some insight into nutrient exchange between symbiont and host. More recently, models for nutrient exchange have been proposed that involve active transport between symbiont and host, and are based primarily on biochemical, cytochemical, and ultrastructural analyses (Gianinazzi-Pearson et al., 1991; Schwab et al., 1991). Although these models vary slightly, they generally involve two-way controlled nutrient transport. The transport processes at the arbuscule-root cell interface are described primarily in terms of a phosphate component:

1. active transport of P into external hyphae by processes typical of VAM fungi and other fungi (Beever and Burns, 1980; Thomson et al., 1990); movement of P

into vacuoles and synthesis of polyphosphate (White and Brown, 1979); transfer of vacuoles into intraradical hyphae by cytoplasmic streaming (Cooper and Tinker, 1981),

2. enzymatic breakdown of polyphosphates (Capaccio and Callow, 1982) and passive or active transport of P down a hypothetical electro-chemical gradient into the apoplastic matrix at the fungus-plant interface (Schwab et al., 1991),

3. active transport into the plant cell, which is driven by a proton motive force derived from plant and fungal ATPase activity and facilitated by existing P-transport proteins in the plasma membrane (Clarkson, 1985; Gianinazzi-Pearson et al., 1991).

In addition, the transport processes are described in terms of a carbohydrate component:

1. passive or active transport of carbohydrates from host into the interfacial matrix, possibly involving a triose P-transporter (as in chloroplasts) and acid phosphatases (Clarkson, 1985; Schwab et al., 1991),

2. active transport into the VAM fungus driven by the same proton motive force and ATPase activity as for the plant P-transport, conversion of carbohydrates into lipids by the fungus to maintain a concentration gradient promoting efflux by the plant.

The major difficulty for confirming such models is our inability to fully characterize the biochemical events occurring at the fungal-plant interface. Hence, it has not been possible to confirm the operation of a triose-P translocater or to account for the unusual event of an outward flux of P from the intraradical hyphae (Clarkson, 1985; Gianinazzi-Pearson, 1991; Schwab et al., 1991).

As mentioned, the topic of nutrient exchange may be integral to the question of how the plant regulates its compatibility with VAM fungi. This topic has recently been reviewed by Koide and Schreiner (1992), who summarized three possible types of regulation.

The first involves control of carbohydrate transfer, as discussed above. Secondly, root exudates, which act as 'signals', may regulate fungal growth. For example, CO_2 (2%) and specific flavonoids, either alone, but more so in conjunction, stimulate spore hyphal extension until apparent depletion of spore reserves. Root colonization has also been shown to promoted by the presence of specific flavonoids, although the mechanism by which this fungal stimulation occurred was not apparent. Finally, plants may regulate the amount of VAM growth by their defense responses (Ward, 1986). Plants have been shown to increase chitinase and peroxidase activities in response to VAM infection; however, such enhanced activity was shown to be transient. The influence of P

nutrition on regulatory 'signals' or plant defense responses has not been previously examined.

The ability of plants to exert control over fungal growth may not necessarily be attributable to one specific mechanism. For example, shading plants decreases photosynthesis, movement of carbohydrates to roots (hence, root exudation or transfer of carbohydrates to the fungus) and growth of the fungus. Fungal growth is decreased because plant growth is decreased and hence, invocation for either an alteration in quality of root exudates or defense response seems unnecessary and inappropriate. On the other hand, high P nutrition increases the plant growth potential, carbohydrate movement to roots and root growth, but decreases an existing infection and apparently causes roots to begin to resist infection. Since the actual level of root carbohydrates would not appear to be limiting for fungal growth, perhaps changes in the type or quantity of regulatory 'signals' related to fungal differentiation (for example, arbuscule formation) occur. Hence, limited carbohydrate access may then deter further growth within roots. Moreover, an increased growth potential by improved P nutrition may enhance the capacity of roots to resist root penetration by external hyphae and thus, operate to decrease fungal spread. Recently, mutants of alfalfa plants that are non-nodulating were demonstrated to also be resistant to infection by two *Glomus* species (Bradbury et al., 1991). The use of such mutants will be extremely useful in future studies to characterize the physiological basis for symbiont - host compatibility.

The Potato

The potato is a crop of world-wide importance, providing sustenance to countless growers (Horton and Anderson, 1992). The value of the potato crop has stimulated much research to define the influence of environmental conditions on plant growth, physiology and yield (Cutter, 1992; Dwelle, 1985; Moorby, 1978). Mineral nutrition has been a particular focus of many studies, as it has a significant impact on plant growth and yield, and moreover, as it is amenable to some regulation (Harris, 1978 and 1992). In this regard, the roots of potato have been demonstrated to be relatively inefficient at recovery of fertilizer-P (Pursglove and Sanders, 1981). Hence, potato plants tend to develop P deficiencies and to produce substandard yields under circumstances in which the level of available P in soil is diminished (MacKay et al., 1988).

Differences between plant species in their efficiency of P uptake have been related to root morphology and mycorrhizal dependency. Although many variables are involved, plants with roots that produce slow growth and few root hairs tend to display a greater

dependency on mycorrhizal fungi than those with rapid root growth and many root hairs (Baylis, 1975; Manjunath and Habte, 1991). It is interesting that potato plants have been described as being both sparsely-rooted (Pursglove and Sanders, 1981) and responsive to VAM fungi under field conditions (Black and Tinker, 1976). Although an influence of VAM fungi on potato growth and P nutrition has been reported (Black and Tinker, 1976), morphological or physiological characterization of interactions between VAM fungi and potato plants has never been documented. Accordingly, the research herein was initiated to further describe the physiological effects of differential P nutrition, especially with regard to P-deficiency stress and the influence of VAM fungi on potato growth and physiology. This study was not designed to assess the potential use of VAM fungi in commercial potato production; however, it is widely recognized that in various regions of the world P availability in soils will limit the yield of potato and many other crops, and the economics of these areas will not allow significant use of fertilizer-P (Abbott and Robson, 1982; Harris, 1992). Further research on plant and mycorrhizal interactions may therefore be worthwhile.

The objectives of the research were as follows:

1. to provide an integrated view of changes in the potato plant's growth, development, mineral accumulation and physiology that occur in response to differential P nutrition, and the influence of the VAM symbiosis on these changes.
2. to characterize morphological and physiological interactions of VAM fungi with roots of P-deficient and relatively P-sufficient potato plants, especially in regard to plant P acquisition and host compatibility.

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Chapter II

Influence of Species of Vesicular-Arbuscular Mycorrhizal Fungi and P Nutrition on Growth, Development and Mineral Nutrition of Potato[1]

Introduction

Phosphorus nutrition exerts a significant influence on plant growth and development, as is readily evident when deficiency occurs. Phosphorus deprivation induces a rapid decline in plant hydraulic conductivity, resulting in an appreciable inhibition of leaf expansion (Radin and Eidenbock, 1984). Axillary bud growth is also very responsive to P nutrition and hence, P-deficiency stress can substantially restrict development of the shoot canopy and photosynthetic surface area (Woolley and Wareing, 1972; Fredeen et al., 1989). Photosynthesis and carbohydrate utilization for shoot growth are significantly hindered when P nutrition is inadequate (Qiu and Israel, 1992), resulting in a shift in photoassimilate and P partitioning, to favor root growth at the expense of shoot growth (Cogliatti and Clarkson, 1983). For the potato (*Solanum tuberosum* L.), adequate P nutrition is critical for tuber development and simultaneously to maintain a high photosynthetic rate during tuber bulking (Nelson et al., 1947). Because the potato has an inherently low root-density and ability to recover fertilizer-P (Pursglove and Sanders, 1981), P deficiency can be a limiting factor to yield in commercial potato production (MacKay et al., 1988).

Many P-deficiency responses of plants have been described, some of which are suggested to be adaptive and to improve plant P nutrition. These include the induction in roots of a P-transport system which exhibits enhanced affinity and transport capacity for P (Cogliatti and Clarkson, 1983); enhanced extracellular and intracellular acid phosphatase activity (Goldstein et al., 1988; Lefebvre et al., 1990); and modifications to glycolysis that alleviate the effects of a P starvation-induced decrease in ATP availability (Duff et al., 1989). While these adaptations may improve P nutrition specifically, P starvation has a rapid, negative influence on the overall mineral nutrition of plants. Root nitrate uptake and translocation to shoots is rapidly diminished (Lee, 1982; Rufty et al., 1990), and similar results have been reported for sulfate (Lee, 1982) and magnesium (Skinner and Matthews, 1990). Such changes in

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selectivity of ion uptake are at least partially responsible for reductions in mineral nutrient accumulation in P deficient plants.

Since P nutrition is linked to uptake and assimilation of other mineral nutrients, it is possible that some of the growth and physiological responses attributed to P deficiency may actually reflect P starvation-induced deficiencies in other essential elements (Skinner and Matthews, 1990). Interpretation of the direct influence of P nutrition on plant morphology and physiology may be further confounded by involvement of a mycorrhizal symbiosis. Uptake of P by plants in P deficient soils is substantially improved by mycorrhizal symbioses (Harley and Smith, 1983). Furthermore, a VAM[2] symbiosis can also increase uptake of other mineral nutrients (Lambert et al., 1979; Kucey and Janzen, 1987; Smith and Gianinazzi-Pearson, 1988), although this latter effect may be through the alleviation of plant P deficiency (Lambert et al., 1979; Lee, 1982). On the other hand, some studies indicate that enhanced Zn and Cu uptake by VAM fungi, and subsequent transfer to hosts, may specifically improve plant growth under Zn or Cu deficient conditions (Kucey and Janzen, 1987). Other influences of a VAM symbiosis on plant development may be totally unrelated to plant mineral physiology (Smith and Gianinazzi-Pearson, 1988).

In previous studies, we demonstrated detrimental effects of P deficiency on growth and physiology of potato grown under tuber-inducing conditions and moreover, characterized some adaptive responses of plants to P-stress (McArthur and Knowles, 1992; McArthur and Knowles, 1993). Inoculation of roots with a VAM fungus produced a low resistance response and substantial infection, thus indicating a high degree of plant compatibility; however, the VAM symbiosis alleviated P deficiency to only a minor extent. This study was initiated to provide an integrated view of changes in the potato plant's growth and development that occur under non-tuber-inducing conditions in response to P-deficiency stress, and the influence of the VAM symbiosis on these changes. By accounting for the effects of P nutrition and the VAM symbiosis on plant P status, and simultaneously describing plant N, K, Mg, Fe, and Zn status, we have characterized both direct and indirect effects of these treatments on mineral physiology of potato at the whole-plant level. Different VAM species were compared for their ability to colonize roots and to produce a growth response in potato.

MATERIALS AND METHODS

Inocula

Inocula for NM and VAM (*Glomus dimorphicum* [Boyetchko and Tewari], *G. intraradices* [Schenck and Smith], and *G. mosseae* [(Nicol. & Gerd.) Gerd. & Trappe])

treatments consisted of soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved 3:1 (v/v) sand:soil medium for 120 d. Pot culture of *G. dimorphicum* was obtained as reported by Boyetchko and Tewari (1986), and *G. intraradices* and *G. mosseae* were obtained from the International Culture Collection of VA Mycorrhizal Fungi at the University of Florida, Gainesville. Plants were grown in 2-L pots and were fertilized weekly with 50 mL of a modified (minus P) Hoagland's solution. Roots of NM clover plants were uninfected, while those of VAM plants were 75% infected, and had many vesicles and chlamydospores. To quantify infection, roots were cut to 1-cm lengths and stored in FAA solution [5% formalin, 5% acetic acid, 45% ethanol (v/v)] until further processing by clearing and staining (Phillips and Hayman, 1970). Percent infection was assessed using the gridline intersect method (Giovanetti and Mosse, 1980) with one hundred counts per slide, and 10 (clover) or 10 (potato [*Solanum tuberosum* L. cv Russet Burbank]) slides per plant.

Plant Growth Conditions and Experimental Design

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface-sterilized with 0.1% (w/v) sodium hypochlorite (10 min) and rinsed thoroughly with distilled H_2O. Single-eye seedpieces (6.0 g) were cut from the mid-region of the tubers and rinsed. After air-drying for 1 h, seedpieces were planted in vermiculite and were sprouted in the dark for twelve d at 27°C (95% RH).

Excised sprouts with attached roots were blocked for sprout-length prior to transplanting onto NM or VAM soil inoculum (100 g fresh weight pot^{-1}) in 25-cm diameter pots (1 sprout pot^{-1}). Each pot contained 7.0 kg (air-dry) of an autoclaved 3:1 (v/v) sand:soil medium (8 µg/g $NaHCO_3$-extractable P). An equal mix of fine and coarse sand was used in the medium, and the soil was a silty clay loam (Malmo Typic haploboroll), with a cation exchange capacity of 0.5 millimhos cm^{-1}. A 2:1 (v/v) water extract of this soil had a pH of 6.2, no detectable P, 33 µg total-N, 40 µg Ca, 11 µg Mg, and 5 µg K (g^{-1} soil). Pots were placed in a growth chamber set at 25/18°C (day/night) with a 16-h photoperiod. A PPFD of 300 µmol m^{-2} s^{-1} was provided by fluorescent and incandescent lights maintained at 10 cm from the shoot tips. In each of the first three weeks, each pot received 100 mL of nutrient solution (40 mM KNO_3; 20 mM $Ca(NO_3)_2$; 20 mM $MgSO_4$; 185 µM H_3BO_3; 36.5 µM $MnCl_3$; 0.3 µM $ZnSO_4$; 1.3 µM $CuSO_4$; 0.065 µM H_2MoO_4; 10 mg/L FeEDDHA [pH 6.0]) with added P. In the fourth week and thereafter, each pot received 100 mL three times per week (30 applications total). Thus, treatments consisted of three levels of P (0.0, 0.5 or 2.5 mM KH_2PO_4), four inocula (NM or one of three VAM species), four harvest dates (0, 28, 56, and 84

DAP) arranged factorially in a randomized complete block design (four blocks). At harvest, leaf number, leaf area, and stem number were recorded. Plant tissues were divided into stems, leaves, tubers and roots and lyophilized for dry weight and mineral analyses. From these variables, L_W/W, R_W/W, S_W/W, T_W/W, LAR (LA/W), RGR (1/W x dW/dt), SAR ($1/R_W$ x dP/dt), SLA (LA/L_W), and ULR (1/LA x dW/dt) were calculated (Hunt, 1982). P-accumulation/P-available was calculated as follows: (the cumulative total of plant P at each harvest date minus initial plant P content at transplanting)/(cumulative total of P supplied at each harvest date, including total $NaHCO_3$-extractable soil P pot^{-1}). RSAR was calculated as SAR /(P-available minus P-accumulation at the previous harvest date).

For the determination of P, K, Mg, Fe, and Zn concentrations, lyophilized plant material was ground through a Wiley mill (40 mesh), and 200 mg was dry-ashed at 500°C for 8 h. Ash was dissolved in 1.0 mL HCl and made up to 10 mL using 0.1 N H_2SO_4. Tissue P was determined by the method of Serrano et al. (1976). Shoot Mg, Fe and Zn concentrations were determined with a Perkin-Elmer Model 4000 atomic absorption spectrophotometer at $A_{202.6}$, $A_{248.3}$, and $A_{213.9}$, respectively. Flame emission spectroscopy with an $A_{766.5}$ was used to determine shoot K levels. To decrease interferences by P and ionization in Mg and K determinations, samples were diluted to produce solutions containing 0.1% La ($LaCl_3 \cdot 6H_2O$) and 0.1% KCl (for Mg), or 0.1% La (for K) (Anon, 1979). For analysis of shoot N, 50 mg of ground tissue was digested in H_2SO_4 containing a catalyst (1:10:100 SeO_2:$CuSO_4$:K_2SO_4), and N determined with an A_{630} by the method of Fawcett and Scott (1960).

Soluble-N and Soluble Carbohydrate Assays

For determination of plant N-compounds and carbohydrate content, 50 mg of ground, lyophilized plant material was extracted (4°C) for 3 min (mortar and pestle) in 5 mL of 50 mM Hepes buffer (pH 7.4). The homogenate was centrifuged (1640g, 4°C) for 30 min and free amino-N, soluble protein-N and NO_3-N were determined on 100, 200 and 50 µL of cold supernatant, respectively. Soluble protein-N was measured by a modified Lowry method (Bensadoun and Weinstein, 1976) with a BSA (15.6% N) standard. Ninhydrin was used to assay free amino-N (Rosen, 1956) with a leucine (10.7% N) standard. Nitrate-N was determined by the methods of Cataldo et al. (1975), with a KNO_3 standard. Reducing sugars were assayed colorimetrically by the methods of Nelson (1944) and Somogyi (1952) using a 100-µL aliquot of extract and a glucose standard. Total soluble carbohydrates were determined on a 50-µL aliquot of supernatant with the phenol-sulfuric acid reagent (Dubois et al., 1956) and a glucose standard.

Statistical Analyses

Growth and physiological data were subjected to analysis of variance and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Percent-infection data were arcsin transformed to achieve homogeneity of variance prior to ANOVA. Based on the results of the ANOVA, regression analysis was used to derive polynomial models for describing the various relationships. In this study, $P_{deviation}$ indicates a significant residual variance that is not explained by a linear model, and with no further trend analysis possible

RESULTS

Development of Root Infection

Root colonization by *G. intraradices* and *G. mosseae* was rapid, as evidenced by a 43% and 40% level of root infection, respectively, at 28 DAP (Fig. 1). At 28 DAP, infected roots showed many penetration points, hyphal coils, and arbuscules, while vesicles were absent. By 84 DAP, percent root infection had doubled, and root vesicles and external clusters of chlamydospores were common. Roots inoculated with *G. dimorphicum* initially had a low rate of infection, but this increased later in the experiment so that differences in level of infection between *G. dimorphicum* and other species averaged only 9% at 84 DAP. Significant differences between species' ability to colonize roots were evident throughout this study, however, and *G. intraradices* produced the most root infection, while *G. dimorphicum* produced the least. No significant interaction between VAM species and abiotic (i.e. exogenous) P level was evident for root infection, and the main effect of increasing abiotic P supply was to decrease percent root infection by an average of 8% and 17% for low-P (0.5 mM) and high-P (2.5 mM) plants, respectively, relative to plants without supplemental P (no-P) (Fig. 1, inset).

Analysis of Plant Growth, Development and Morphology

Plant growth during the first 28 DAP was unaffected by differential P nutrition, probably reflecting the initial small size of plants relative to the P available. By 56 DAP, however, NM plants fertilized with no-P (0.0 mM) or low-P had accumulated 39% and 32% less dry matter, respectively, than plants grown with high-P (Fig. 2). By 84 DAP, no-P plants and low-P NM plants had 65% and 45% less dry weight, respectively, than high-P plants. Inoculation of roots with a VAM fungus provided a distinct advantage to plants grown with no-P or low-P, as demonstrated by an average 73% and 43% greater plant dry weight, respectively, at 84 DAP, compared to NM plants with the same

Figure II-1. Main effect of time on percent root infection by *Glomus dimorphicum* (▲), *G. intraradices* (□), or *G. mosseae* (●) for potato plants (averaged for plants grown with 0.0 mM, 0.5 mM, or 2.5 mM P) inoculated with VAM clover pot culture. *F*-values for *G. dimorphicum versus* (average of *G. intraradices* and *G. mosseae*) and *G. mosseae versus G. intraradices* were significant at the 0.01 level. The interaction of $Time_{quadratic}$ x *G. dimorphicum versus* (average of *G. intraradices* and *G. mosseae*) was significant at the 0.01 level. Inset: Main effect of increasing abiotic P supply on percent root infection, averaged for VAM species (●). *F*-value for the linear trend was significant at the 0.01 level.

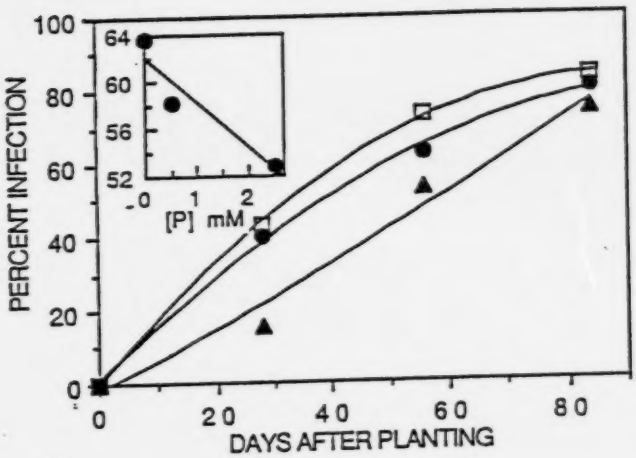
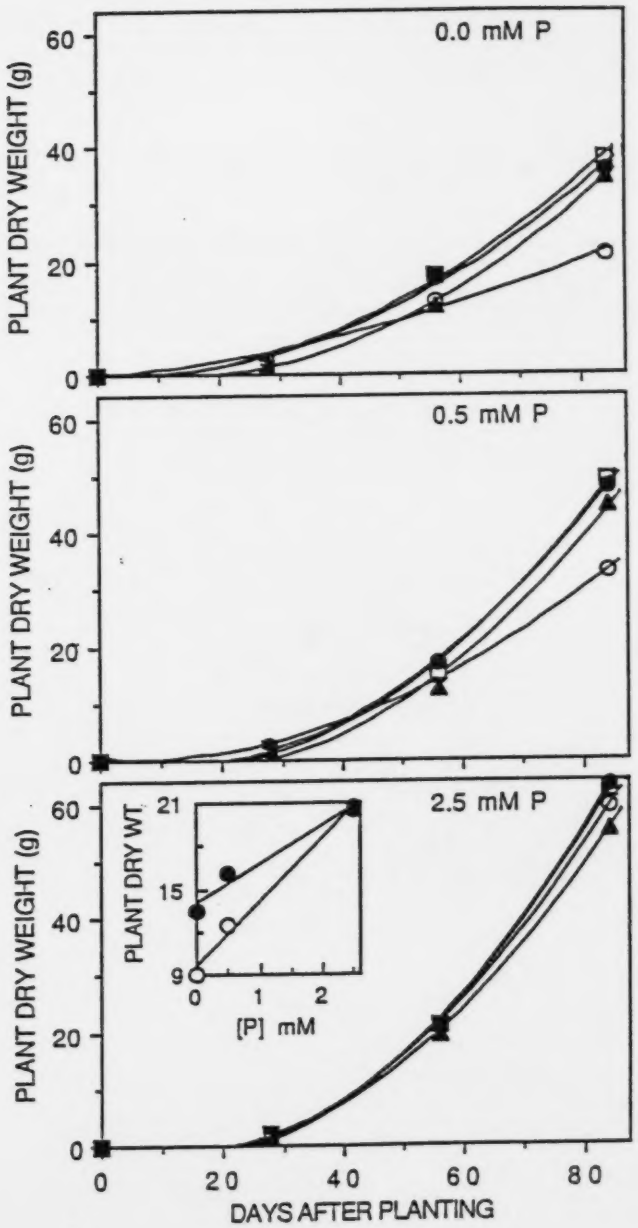

PERCENT INFECTION
100
80
60
40
20
0
0
20
40
60
80
DAYS AFTER PLANTING
64
60
56
52
0
1
2
[P] mM

Figure II-2. Time course of dry matter accumulation of NM (○) and VAM plants (*G. dimorphicum* ▲; *G. intraradices* □; *G. mosseae* ●) grown with 0.0 mM, 0.5 mM, or 2.5 mM P for 84 d. *F*-values for the main effects of VAM, *G. dimorphicum versus* (average of *G. intraradices* and *G. mosseae*), and $Time_{quadratic}$, and the interaction of VAM x P_{linear} x $Time_{quadratic}$ were significant at the 0.01 level. Inset: Main effect of abiotic P supply on dry matter accumulation for NM plants and the average of VAM plants (●). *F*-values for P_{linear}, and the interaction of VAM x P_{linear} were significant at the 0.01 level.


0.0 mM P
0.5 mM P
2.5 mM P
PLANT DRY WEIGHT (g)
PLANT DRY WT
[P] mM
DAYS AFTER PLANTING
0
20
40
60
80
1
2
9
15
21

abiotic P supply. The amount by which plant growth was stimulated by VAM species depended upon the rate of root infection (Abbott and Robson, 1982) and hence, *G. intraradices* and *G. mosseae*, which more rapidly colonized roots, increased plant dry weight at 84 DAP by an average of 10% more than did *G. dimorphicum* ($P < 0.01$). When P was sufficient (Fig. 2C, inset), no significant influence of a VAM symbiosis on dry mass accumulation was observed, in spite of an average 53% level of root infection (Fig. 1, inset).

Phosphorus nutrition and the VAM symbiosis exerted strong influences over plant development and morphology. The polygonal diagrams in Figure 3 summarize the effects of VAM fungi and abiotic P supply on plant yield components at 28, 56, and 84 DAP. Results of the ANOVA for treatment effects on each yield component depicted in Figure 3 appear in Table I. Positive main effects of increasing abiotic P supply, time, and the VAM symbiosis were evident for each plant yield component. Multiple interactions between treatments characterized a relatively large benefit from the VAM symbiosis for no-P and low-P plants, but a relatively minor effect of the VAM symbiosis on high-P plants.

Growth and shoot canopy development were restricted by inadequate P nutrition by 56 DAP and substantially so between 56 and 84 DAP. A primary reason for the diminished total leaf area was a significant restriction of lateral bud growth associated with low P nutrition (Fig. 3). Expansion of photosynthetic surface area for Russet Burbank plants depends upon lateral branch growth after the primary shoot flowers (between 28 and 56 DAP in our study). Between 56 and 84 DAP, no-P NM plants increased their branch number by 34%, whereas high-P NM plants nearly doubled their branch number. Leaf number for no-P NM plants also increased by 32% from 56 to 84 DAP, however, this potential contribution to total leaf area was partially offset by an 18% reduction in area per leaf for the same period ($P < 0.01$). As a result of this inhibition of growth and devlopment, no-P NM plants had a 75 and 80% lower total leaf area and leaf dry weight, respectively, than high-P NM plants at 84 DAP. Stem (including tubers) and root growth were somewhat less restricted by inadequate P nutrition, as indicated by the 60 and 36% lower dry weights, respectively, at 84 DAP for no-P plants than for high-P plants.

Comparing the shape and relative areas encompassed by the polygonal diagrams for VAM plants with those for NM plants demonstrates the advantage of the VAM symbiosis to plant growth and development under P-limiting conditions (Fig. 3). By 84 DAP, VAM plants grown without any supplemental P had a dry weight equivalent to that of NM plants grown with low-P (0.5 mM) and moreover, no-P VAM plants had a 24% higher

Figure II-3. Polygonal diagrams describing growth, development, and overall plant morphology of NM and VAM potato plants grown with 0.0 mM, 0.5 mM, or 2.5 mM P, and harvested at 28, 56, and 84 DAP. Axes are defined on the 2.5 mM P polygonal diagrams and stem dry weight includes tuber dry weight. A summary of the statistical analysis for each yield component is presented in Table I. Data are pooled for three VAM species.

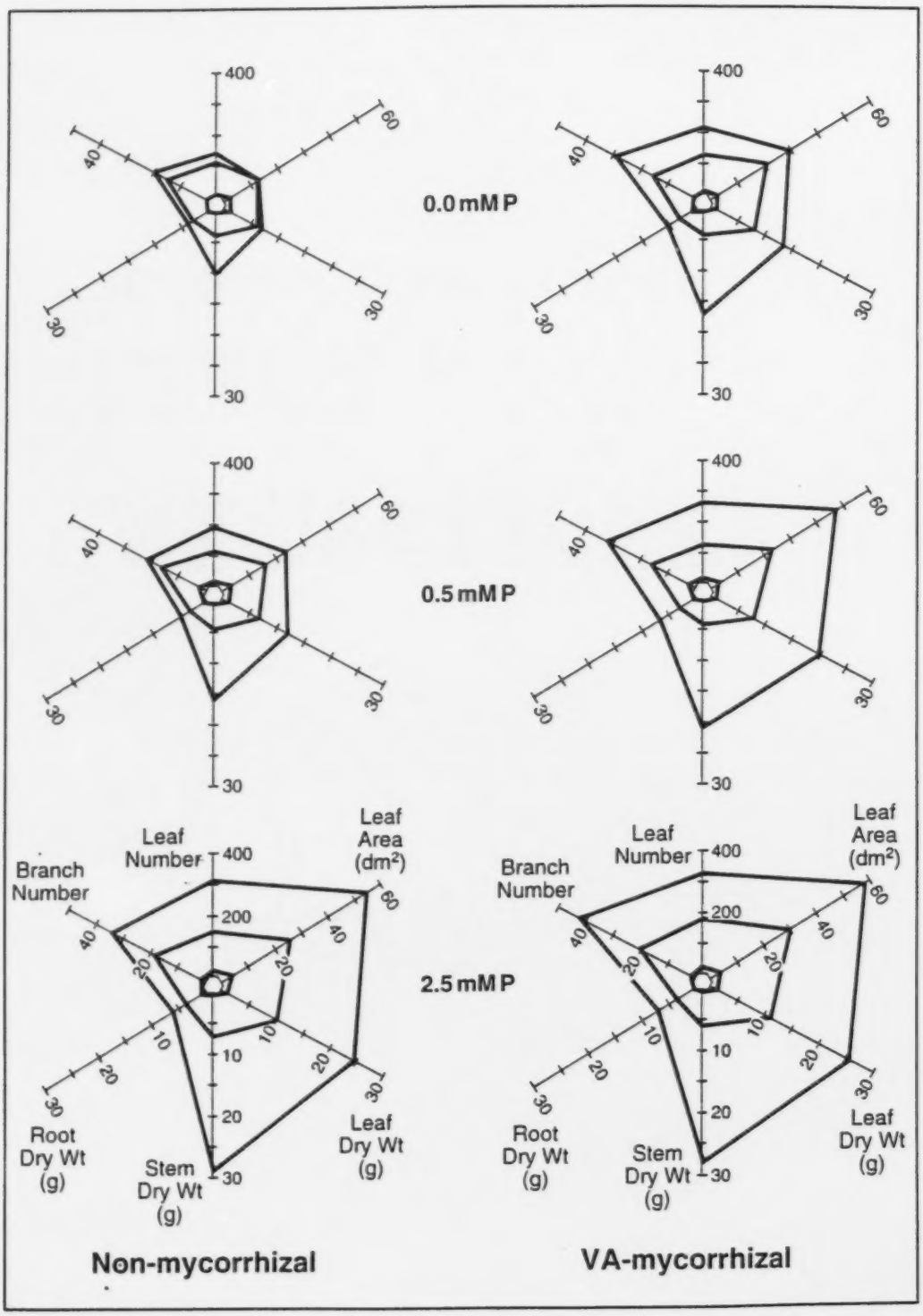

0.0 mM P
0.5 mM P
2.5 mM P
Leaf Number
Leaf Area (dm²)
Branch Number
Root Dry Wt (g)
Stem Dry Wt (g)
Leaf Dry Wt (g)
Non-mycorrhizal
VA-mycorrhizal

Table I. *Partial Summary of Analyses of Variance for Yield Components of Non-mycorrhizal (NM) and Vesicular-arbuscular Mycorrhizal (VAM) Potato Plants Grown with Various Levels of P*

Plants were inoculated with NM or VAM (*Glomus dimorphicum, G. intraradices* or *G. mosseae*) clover pot cultures, and fertilized with a complete nutrient solution containing 0.0 mM, 0.5 mM, or 2.5 mM P. Plants were harvested 28, 56, and 84 DAP. Data are presented in Figure 3

Source of Variation	Branch Number	Leaf Number	Leaf Area	Leaf Dry Wt	Stem[a] Dry Wt	Root Dry Wt	Plant Dry Wt
VAM	0.01[b]	0.01	0.01	0.01	0.01	0.01	0.01
P	L	L	L, D	L, D	L	L, D	L, D
VAM X P_L	ns	0.10	0.01	0.01	0.01	ns	0.01
VAM X $Time_Q$	0.01	0.05	0.01	0.01	0.01	0.01	0.01
P_L X $Time_Q$	0.01	0.01	0.01	0.01	0.01	0.01	0.01
VAM X P_L X $Time_Q$	ns	0.01	0.01	0.01	0.01	ns	0.01
Gi and *Gm* > *Gd*	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Gi > *Gm*	-	-	-	-	0.05	-	-

[a]Includes tuber dry wt.

[b]Significance levels or significant trend (L = linear, Q = quadratic, D = deviations with $P < 0.05$) for indicated sources of variation (ns = not significant).

total leaf area than low-P NM plants. Furthermore, low-P VAM plants were comparable to high-P NM plants in terms of overall growth and development. For high-P plants, however, the influence of the VAM symbiosis on leaf number, stem dry weight, and leaf dry weight was almost negligible, although, total leaf area, branch number and root dry weight were marginally higher for VAM plants than for NM plants.

Relative to roots, dry matter accumulation in leaves and stems was highly responsive to P nutrition. For each 1 mM [P] increase in the nutrient solution supplied to NM plants during growth, dry weights of stems and leaves increased by 7.1 and 6.6 g plant^{-1}, respectively at 84 DAP (r = 0.99). In contrast, the root response to increasing P was only 0.7 g plant^{-1} (r = 0.92). These differential responses resulted in a lower S_W/W ratio with low P nutrition and, conversely, a higher R_W/W ratio (Table II). Tuber dry mass made a relatively minor contribution to total plant dry mass (T_W/W) under our growth conditions and, although tuber growth was enhanced by increasing abiotic P supply, VAM plants were less responsive than NM plants in this regard (VAM x P_{linear}, $P < 0.05$). Tuberization occurred earlier in NM plants (before 56 DAP) than in VAM plants (after 56 DAP) and possibly for this reason, tuber dry weight averaged over abiotic P levels was 52% lower at 84 DAP for VAM plants than for NM plants. This altered partitioning of dry matter by the VAM symbiosis favored a greater allocation of photoassimilates to shoot growth.

Plant growth analysis demonstrated that P nutrition influenced both the net assimilatory capacity of leaf biomass and plant dry matter partitioning (Table III). The ULR, an index of the productive efficiency of leaves, declined linearly (r = 0.91) with decreasing abiotic P level for NM plants. Similarly, LAR for NM plants was lower for no-P or low-P plants than for high-P plants. Reductions in both SLA and L_W/W with decreasing abiotic P level showed that the relative leaf area produced by NM plants was restricted by both diminished leaf expansion and allocation of dry mass to leaf growth. Since RGR = ULR x LAR, and LAR = SLA x L_W/W, these results characterize the significant negative influence of inadequate P nutrition on the RGR of potato plants (Table III).

As expected, VAM plants maintained a higher average RGR than NM plants (Table III), although a highly significant VAM x P_{linear} interaction indicated that differences between NM and VAM plants were diminished by increasing abiotic P supply. At 2.5 mM P, NM plants had an RGR equivalent to that of VAM plants. A major factor in the VAM-stimulated increase in RGR under no-P or low-P conditions was an average 18% increase in LAR for VAM plants, resulting from a combination of enhanced leaf expansion (SLA) and relatively greater allocation of plant dry matter to leaf growth (L_W/W).

Table II. *Relative Dry Matter Allocation of Non-mycorrhizal (NM) and Vesicular-Arbuscular Mycorrhizal Potato Plants Grown with Various Levels of Abiotic P Supply*

Plants were inoculated with NM or VAM (*Glomus dimorphicum, G. intraradices* or *G. mosseae*) clover pot cultures, and fertilized with a complete nutrient solution containing 0.0 mM, 0.5 mM, or 2.5 mM P. The data presented is for 84 DAP only and VAM data is the average for three species.

Treatment		S_W/W[a]	R_W/W	T_W/W
[P] mM	VAM	%	%	%
0.0	-	79.7	17.6	2.8
	+	85.2	14.7	0.6
0.5	-	80.9	15.0	4.1
	+	83.7	13.9	2.5
2.5	-	82.5	9.9	7.7
	+	85.7	11.3	3.0
VAM[b]		0.01[c]	ns	0.01
P		L	L	L
VAM X P_L		ns	0.10	0.05
VAM X P_D		ns	ns	ns

[a] S_W/W, shoot:plant dry wt ratio; R_W/W, root:plant dry wt ratio; T_W/W, tuber:plant dry wt ratio.
[b]Sources of variation (L = linear trend, D = deviations).
[c]Significance levels or significant trend (L = linear with $P < 0.05$) for indicated sources of variation (ns = not significant).

Table III. *Growth analysis of Non-mycorrhizal (NM) and Vesicular-arbuscular Mycorrhizal (VAM) Potato Plants Grown with Various Levels of abiotic P-Supply*

Plants were inoculated with NM or VAM (*Glomus dimorphicum, G. intraradices* or *G. mosseae*) clover pot cultures, and fertilized with a complete nutrient solution containing 0.0 mM, 0.5 mM, or 2.5 mM P. Data was pooled from plants harvested at 28, 56, and 84 DAP and the VAM data is the average for three species. Growth indices were calculated from the data presented in Figures 2 and 3

Treatment		ULR[a]	LAR	SLA	L_W/W	RGR
[P] mM	VAM	*mg cm^{-2} d^{-1}*	*cm^2 mg^{-1}*	*cm^2 mg^{-1}*		*g g^{-1}d^{-1}*
0.0	-	0.51	0.11	0.23	0.36	0.059
	+	0.50	0.13	0.26	0.38	0.065
0.5	-	0.55	0.11	0.24	0.36	0.063
	+	0.49	0.14	0.27	0.40	0.068
2.5	-	0.58	0.12	0.25	0.39	0.070
	+	0.51	0.14	0.26	0.40	0.071
VAM[b]		0.01[c]	0.01	0.01	0.01	0.01
P		L	L, D	D	L	L, D
VAM X P_L		0.10	ns	ns	0.10	0.01
VAM X P_D		ns	ns	ns	ns	ns
Gi and *Gm* > *Gd*		ns	ns	ns	ns	0.01

[a] ULR, unit leaf rate; LAR, leaf area ratio; SLA, specific leaf area; L_W/W, leaf:plant dry wt ratio; RGR, relative growth rate.
[b] Sources of variation (L = linear trend, D = deviations); [c]Significance levels or significant trend (L = linear, D = deviations with $P < 0.05$) for indicated sources of variation (ns = not significant).

Conversely, the ULR for VAM plants was lower than that for NM plants, thus indicating that the photosynthetic surface area of VAM plants was somehow less efficient in producing dry weight gain than that of NM plants. An increase in root respiration of VAM plants compared to NM plants has been observed (Baas et al., 1989) and this could at least partly account for the significantly lower ULR for VAM plants than for comparable NM plants.

P Acquisition

The trend in whole-plant P accumulation depended on P nutrition and the VAM symbiosis (Fig. 4). By 84 DAP, no-P and low-P NM plants had accumulated 76% and 55% less P, respectively, than high-P NM plants. Greater P accumulation with increasing abiotic P supply was associated with a linear increase in SAR for P (SAR = 0.13[P] + 0.44, r = 0.99). The SAR declined with time for no-P and low-P plants, although for high-P plants, a decrease was evident only at the last harvest date (Fig. 4, insets). For a pot study, such results likely reflect the greater overlap of root absorbing surfaces with time, and indicate depletion of soil P for no-P and low-P plants (Sanders et al., 1977).

Differences between NM and VAM plants for P accumulation and SAR were initially negligible (Fig. 4), reflecting relatively high soil-P levels and low levels of VAM infection early in the study. However, differences in P status between NM and VAM plants were evident by 56 DAP at all levels of abiotic P. At 84 DAP, VAM plants grown with no-P, low-P, or high-P had accumulated an average of 115%, 71%, and 34% more P, respectively, than comparably-fertilized NM plants. It is of interest that, although the relative contribution of the VAM fungi was decreased by high-P, the actual increment in milligrams P from VAM fungi marginally increased with abiotic P supply ($P < 0.01$). The basis for higher P accumulation by VAM plants was a 63% higher SAR for VAM roots than for NM roots, when averaged over P levels for the last two harvest dates (Fig. 4, insets). Species differed significantly in their contribution to plant P accumulation; *G. intraradices* increased P uptake the most and *G. dimorphicum* the least ($P < 0.01$).

In spite of the small amount of total P in P-stressed plants, the proportion of available P accounted for by P accumulation was significantly higher than that for high-P NM plants. No-P NM plants had a ratio of P-accumulated/P-available at 84 DAP that was 25% greater than that of high-P NM plants (Fig. 5). Comparison of the RSAR for NM plants at 28 DAP indicates that the flux of P into plants from the pool of available P initially was highest for no-P NM plants, but declined over the harvest interval to a

Figure II-4. Time course of P accumulation for NM (○) plants and VAM plants (*G. dimorphicum* ▲ ; *G. intraradices* □ ; *G. mosseae* ●) grown with 0.0 mM, 0.5 mM, or 2.5 mM P for 84 d. *F*-values for the main effects of VAM, *G. dimorphicum versus* (average of *G. intraradices* and *G. mosseae*), *G. mosseae versus G. intraradices*, P_{linear}, $Time_{quadratic}$ and the of interaction VAM x $Time_{quadratic}$ were significant at the 0.01 level. Insets: Time course of SAR for P for NM plants and the average of VAM (●) plants. *F*-values for $Time_{linear}$, $Time_{deviation}$, VAM, P_{linear}, and the interaction of VAM x $Time_{deviation}$ were significant at the 0.01 level.

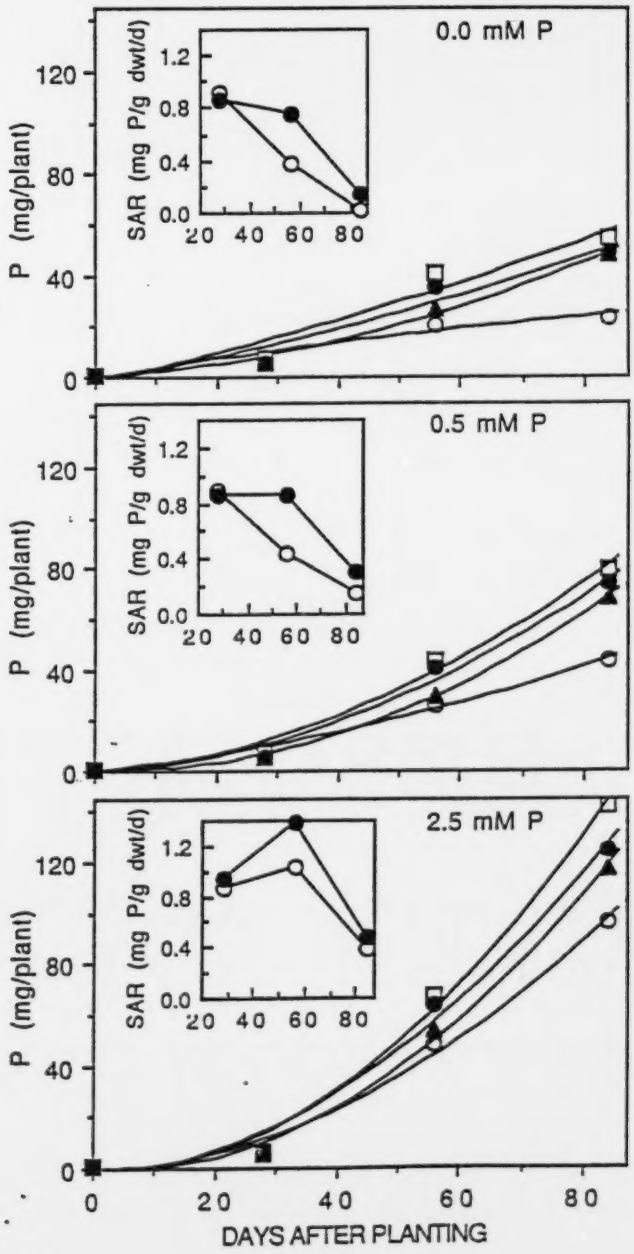
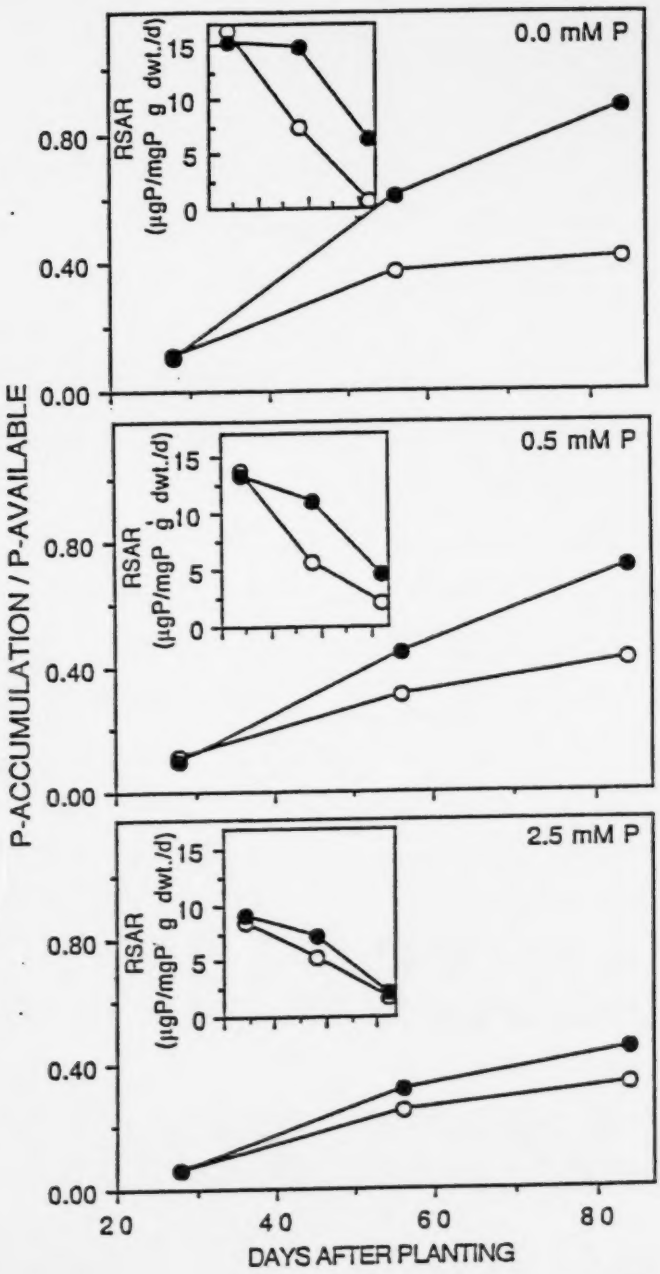

0.0 mM P
0.5 mM P
2.5 mM P
P (mg/plant)
SAR (mg P/g dwt/d)
DAYS AFTER PLANTING
0
40
80
120
0.0
0.4
0.8
1.2
20
40
60
80

Figure II-5. Time course of P-accumulation/P-available for NM (○) plants and the average of VAM plants (●) grown with 0.0 mM, 0.5 mM, or 2.5 mM P for 84 d. *F*-values for the main effects of P_{linear}, VAM, $Time_{linear}$, $Time_{deviation}$ and the interaction of VAM x P_{linear} x $Time_{deviation}$ were significant at the 0.01 level. Insets: Time course of RSAR for P for NM and VAM plants. *F*-values for $Time_{linear}$, $Time_{deviation}$, VAM, P_{linear}, and the interaction of VAM x P_{linear} x $Time_{deviation}$ were significant at the 0.01, 0.01, 0.01, 0.01, and 0.10 level, respectively.


0.0 mM P
0.5 mM P
2.5 mM P
RSAR
(µgP/mgP g dwt./d)
15
10
5
0
P-ACCUMULATION / P-AVAILABLE
0.00
0.40
0.80
20
40
60
80
DAYS AFTER PLANTING

value less than that of high-P NM plants (Fig. 5, insets). This decline in RSAR with time for P-stressed plants likely reflects the depletion of soil P which roots had ready access to. On the other hand, the slow decline in the RSAR for high-P NM plants was more likely due to the enhanced root growth producing root surface overlap and, thus, a reduction in absorption efficiency.

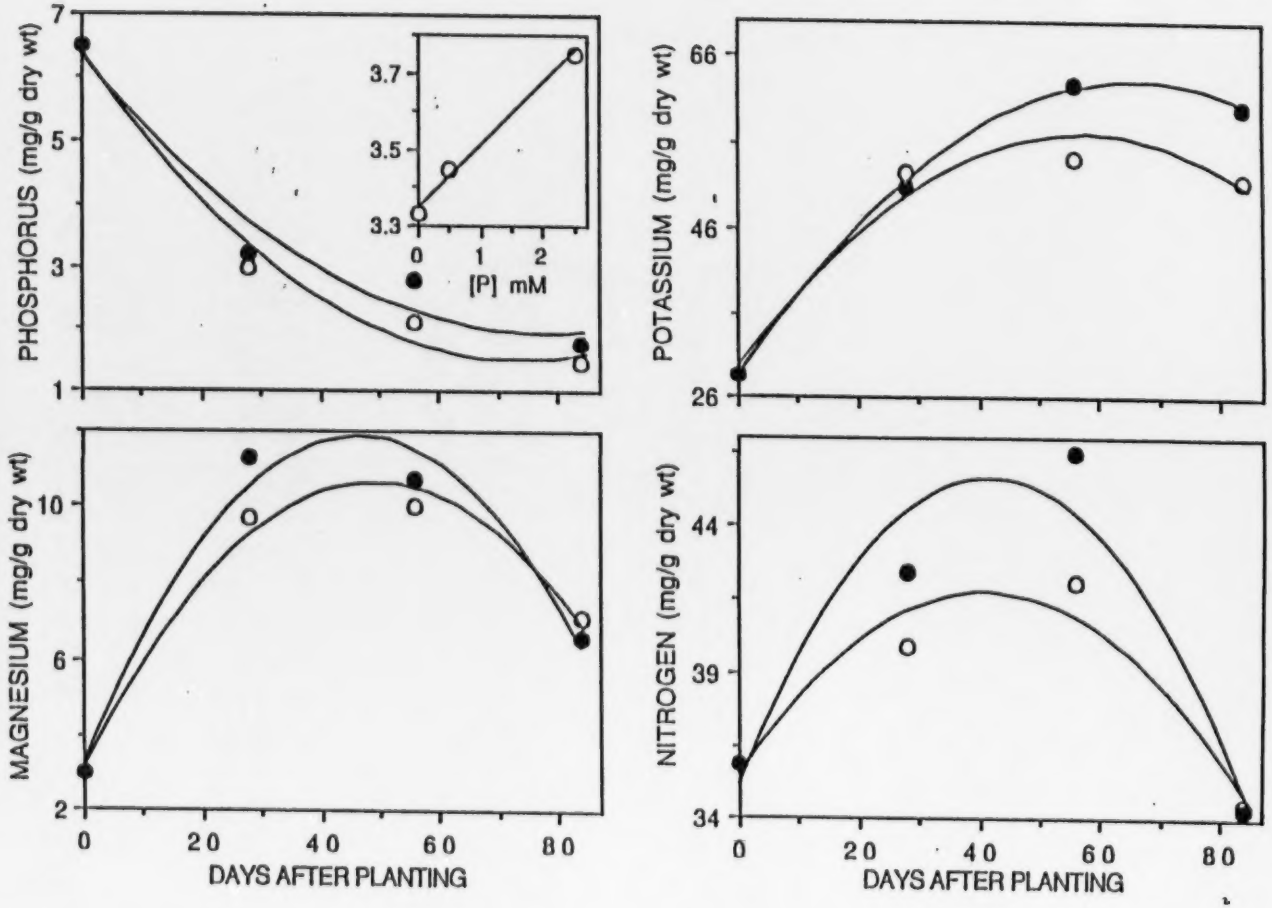
The P-accumulation/P-available ratio demonstrated that no-P and low-P VAM roots were twice as efficient in absorbing available P than no-P or low-P NM roots (Fig. 5). The relative benefit of the VAM symbiosis to P acquisition was inversely related to increasing abiotic P supply, but was still evident for high-P plants. The RSAR of VAM roots declined more slowly with time than that of NM roots ($P < 0.01$) and, at 84 DAP, the RSAR of no-P VAM plants was 9.3 and 4 times greater, respectively, than that of no-P and high-P NM plants (Fig. 5, insets). For high-P plants, the difference between NM and VAM plants in RSAR was negligible indicating that any improvement to efficiency of P uptake associated with the VAM symbiosis was of minor importance.

Shoot Mineral Physiology

Although plants accounted for an increasing proportion of available P over time, P uptake could not match dry matter accumulation, as was evident by the declining concentration of P in shoots (Fig. 6A). P concentration declined most rapidly between 0 and 28 DAP, reflecting rapid early growth in spite of limited P resources (seedpiece reserves were excised from sprouts at transplanting) and a relatively undeveloped root system. For each 1 mM [P] increase, shoot-P increased by 0.16 mg g^{-1} dry weight over the 84-d growth period ([shoot-P] = 0.16 [P] + 3.35, $r = 0.99$) (Fig. 6, inset). The main effect of the VAM symbiosis was to increase shoot-P concentration, with a marginally significant difference between NM and VAM plants evident as early as 28 DAP ($LSD_{0.05}$ = 0.13 mg g^{-1} dry weight).

Whereas shoot-P concentration was highest at the transplant stage; K, Mg, and N (mg g^{-1} dry wt) increased from transplanting to about 56 DAP indicating rapid mineral nutrient uptake in relation to dry matter accumulation (Fig. 6). These levels then declined during the period of greatest dry mass accumulation, which is in agreement with previous reports on the mineral nutrition of potato (Harris, 1978). In contrast to shoot-P concentration; K, Mg, and N levels did not show any significant response to abiotic P supply. However, a highly significant main effect of VAM and a VAM x $Time_{quadratic}$ interaction demonstrated that VAM plants had higher shoot concentrations of K, Mg, and N than NM plants on two of three harvests dates after inoculation, and as early as 28 DAP.

Figure II-6. Interactions between the effects of VAM fungi and time on shoot phosphorus, potassium, magnesium, and nitrogen concentrations of potato plants harvested at 0, 28, 56, and 84 DAP. Data are for NM (○) plants and the average of VAM (●) plants. For P, *F*-values for the main effects of VAM, $Time_{quadratic}$, and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01 level. Inset: Main effect of abiotic P supply on shoot-P concentration. *F*-value for the linear trend was significant at the 0.01 level. For K, *F*-values for the main effects of VAM and $Time_{quadratic}$ were significant at the 0.01 level. For Mg, *F*-values for the main effects of VAM, $Time_{quadratic}$, and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01 level. For N, *F*-values for the main effects of VAM, $Time_{quadratic}$, and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01 level.


PHOSPHORUS (mg/g dry wt)
7
5
3
1
3.7
3.5
3.3
0
1
2
[P] mM
POTASSIUM (mg/g dry wt)
66
46
26
MAGNESIUM (mg/g dry wt)
10
6
2
0
20
40
60
80
DAYS AFTER PLANTING
NITROGEN (mg/g dry wt)
44
39
34
0
20
40
60
80
DAYS AFTER PLANTING

Although the level of shoot-N was not altered by P nutrition, concentrations of soluble protein-N, free amino-N and nitrate-N were increased by inadequate P nutrition (Table IV). Higher levels of such soluble-N compounds in shoots reflect the slow growth and nitrate assimilation, relative to N accumulation, by P deficient plants. Less capacity for nitrate assimilation was evident since reduced-N soluble-N^{-1} was significantly lower in shoots of no-P or low-P plants than in high-P plants. Insoluble-N in shoots increased linearly ($P < 0.01$) with increasing abiotic P supply, possibly indicating increased incorporation of soluble-N into nucleic acids, imino acids and structural N-compounds, which were not detected by our assays. No-P and low-P VAM plants had higher concentrations of free amino-N than comparably-fertilized NM plants, although no difference between NM and VAM plants was observed with high-P nutrition (Table IV). Most of the difference in the concentration of total shoot-N between VAM and NM plants was attributable to an enhanced level of insoluble-N in VAM plants. Since an enhanced level of insoluble-N in shoots was also associated with high P nutrition, the VAM effect on insoluble-N was probably mediated indirectly through the improved plant P status afforded by the VAM symbiosis.

Shoot-Fe concentration ($\mu g\ g^{-1}$ dry weight) declined with time, and was not altered by VAM or abiotic P supply (Fig. 7). While such a trend may indicate a developing deficiency, comparison of these shoot-Fe levels with published values for potato (Harris, 1978), indicated that Fe was not likely limiting to plant growth in our study. The effects of P nutrition and VAM infection on Zn concentrations of shoots were in contrast to those on shoot-P. Shoot-Zn concentration fell significantly as abiotic P supply increased (Fig. 7, inset), and was 18% less for VAM plants, compared with NM plants, when averaged over the study interval (Fig. 7). Shoot-Zn decreased by 3.5 $\mu g\ g^{-1}$ dry weight with each 1 mM [P] increase ([shoot-Zn] = -3.5[P] + 60.1, $r = 0.99$) when averaged over the harvest interval. Increasing the abiotic P supply possibly produced interactions between Zn and Fe oxides in the soil, rendering Zn less available (Loneragan et al., 1979).

Although P nutrition had no effect on the concentrations of N, K, Mg, or Fe in shoots, it strongly influenced total mineral accumulation, as depicted in the polygonal diagrams of Figure 8. Each polygonal diagram summarizes the content of N, P, K, Mg, Fe, and Zn in shoots of potato plants at 28, 56, and 84 DAP for NM and VAM plants grown with a particular abiotic P level. Table V summarizes treatment effects for each element as characterized by ANOVA. Comparison of polygonal diagrams for no-P NM plants with low-P or high-P NM plants indicated that mineral nutrient accumulation was restricted by P-stress, and maximally so between 56 and 84 DAP. Shoot content of P, K, Mg, N, Fe

Table IV. *Effect of Vesicular-Arbuscular Mycorrhizal (VAM) Fungi and P Nutrition on N-Pools in Shoots of Potato Plants*

Plants were inoculated with non-mycorrhizal or VAM (*Glomus dimorphicum*, *G. intraradices*, or *G. mosseae*) clover pot cultures, and fertilized with a complete nutrient solution with 0.0 mM, 0.5 mM, or 2.5 mM P. Data was pooled for plants harvested at 28, 56, and 84 DAP, and VAM data is the average for three species

Treatment		NO_3-N	Soluble Amino-N	Soluble Protein-N	Insoluble-N	Reduced-N[a] Soluble-N^{-1}
[P] mM	VAM	*mg g^{-1} shoot dry wt*				
0.0	-	9.7	2.5	9.7	17.6	0.56
	+	10.0	2.9	9.2	19.3	0.54
0.5	-	9.6	2.3	9.0	17.9	0.55
	+	10.2	2.8	9.0	19.0	0.54
2.5	-	9.0	2.4	8.6	18.3	0.56
	+	8.4	2.4	9.0	20.9	0.58
VAM[b]		ns[c]	0.01	ns	0.01	ns
P		L	L	L	L	L
VAM X P_L		ns	0.10	0.05	ns	ns
VAM X P_D		ns	ns	ns	ns	ns
Gi and *Gm*>*Gd*		ns	0.05	ns	ns	ns

[a](soluble amino-N+protein-N)/(total soluble-N) ratio for shoots.
[b] Sources of variation. [c] Significance levels or trend (L = linear, D = deviations with $P < 0.05$) for indicated sources of variation (ns = not significant).

Figure II-7. Change in shoot iron and zinc concentrations of NM (○) and VAM (●) potato plants harvested at 0, 28, 56, and 84 DAP. Data are pooled for three VAM species. For Fe, *F*-value for the main effect of $Time_{quadratic}$ was significant at the 0.01 level. For Zn, *F*-values for the main effects of VAM and $Time_{quadratic}$, and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01 level. Inset: Main effect of abiotic P supply on shoot-Zn concentration. *F*-value for the linear trend was significant at the 0.01 level.

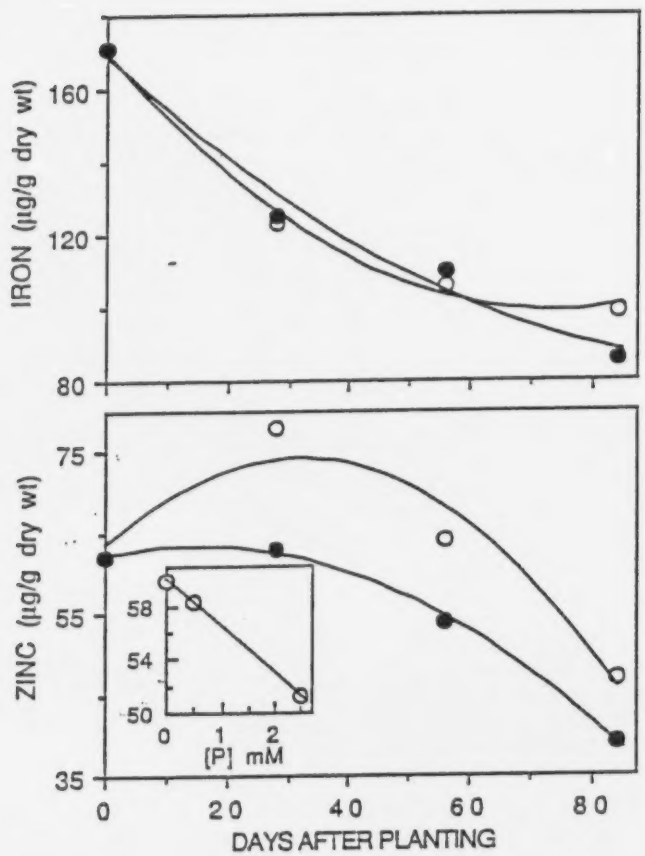

IRON (µg/g dry wt)
160
120
80
ZINC (µg/g dry wt)
75
55
35
58
54
50
0
1
2
[P] mM
0
20
40
60
80
DAYS AFTER PLANTING

Figure II-8. Polygonal diagrams describing mineral nutrient accumulation in shoots of NM and VAM potato plants grown with 0.0 mM, 0.5 mM, or 2.5 mM P, and harvested at 28, 56, and 84 DAP. Data are pooled for three VAM species. Axes are defined on the 2.5 mM P polygonal diagrams. A summary of the statistical analysis for each mineral nutrient is presented in Table V.

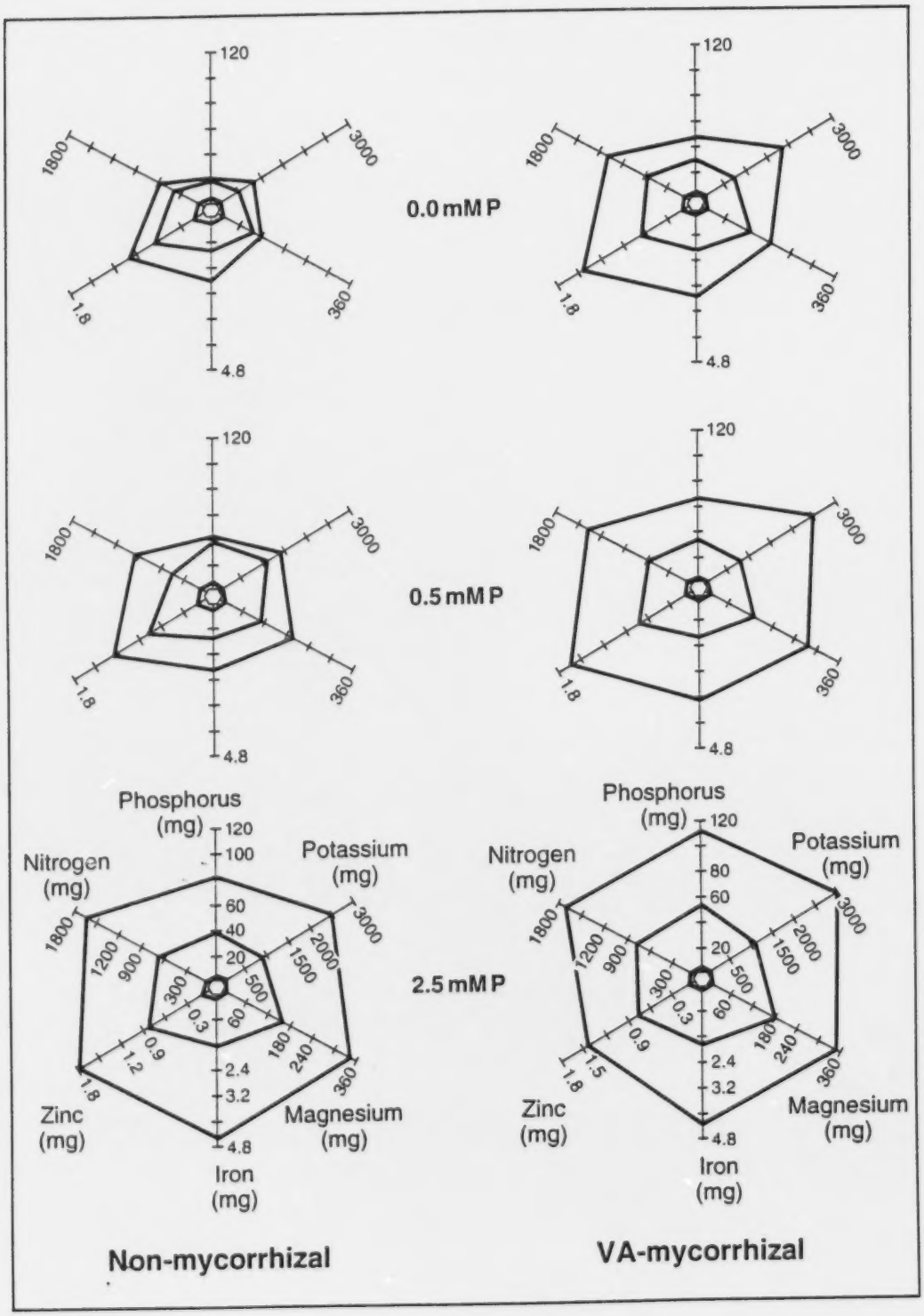

0.0 mM P
0.5 mM P
2.5 mM P
Phosphorus (mg)
Potassium (mg)
Magnesium (mg)
Iron (mg)
Zinc (mg)
Nitrogen (mg)
Non-mycorrhizal
VA-mycorrhizal

Table V. *Partial Summary of Analyses of Variance for Mineral Elements Accumulated in Shoots of Non-mycorrhizal (NM) and Vesicular-arbuscular Mycorrhizal (VAM) Potato Plants Grown with Various Levels of Abiotic P-supply*

Plants were inoculated with NM or VAM (*Glomus dimorphicum, G. intraradices or G. mosseae*) clover pot cultures, and fertilized with a complete nutrient solution containing 0.0 mM, 0.5 mM, or 2.5 mM P. Plants were harvested 28, 56, and 84 DAP. Data are presented in Figure 7

Source of Variation	N	P	K	Mg	Fe	Zn	Total Plant P
VAM	0.01[a]	0.01	0.01	0.01	0.01	ns	0.01
P	L, D	L, D	L, D	L, D	L	ns	L, D
VAM X P_L	0.01	ns	0.01	0.01	0.05	0.01	ns
VAM X $Time_Q$	0.01	0.01	0.01	0.05	ns	0.05	0.01
P_L X $Time_Q$	0.01	0.01	0.01	0.01	0.01	ns	0.01
VAM X P_L X $Time_Q$	0.01	ns	0.01	0.01	0.10	0.01	ns
Gi and *Gm* > *Gd*	0.01	0.01	0.01	0.01	0.05	ns	0.01
Gi > *Gm*	0.01	0.01	0.01	-	-	ns	0.01

[a]Significance levels or significant trends (L = linear, Q = quadratic, D = deviations with $P < 0.05$) for indicated sources of variation (ns = not significant).

and Zn at 84 DAP was 74%, 68%, 66%, 64%, 56% and 45% lower, respectively, for NM plants grown with no supplemental P than for plants grown with 2.5 mM P.

Comparison of the polygonal figures for VAM plants with those for NM plants shows that even partial alleviation of P deficiency by the VAM symbiosis markedly improved mineral nutrient accumulation in shoots (Fig. 8). Similar to the yield variables shown in the polygons of Figure 3, at 84 DAP, no-P VAM plants matched or even exceeded the mineral nutrient accumulation of low-P NM plants. Uptake of nutrients was not limited by P-stress for plants grown with 2.5 mM P; however, high-P VAM plants demonstrated enhanced accumulation of not only P, but also K ($LSD_{0.05}$ = 156 mg shoot^{-1}) in shoots. Although the VAM symbiosis enhanced shoot-Zn content for P-deficient plants, high-P VAM plants paradoxically had a lower shoot-Zn content (VAM x P_{linear}, $P < 0.01$) than high-P NM plants.

DISCUSSION

Growth and development of potato plants were demonstrated to be very responsive to P nutrition. Moreover, our results characterized the progressive development of P deficiency between 28 and 84 DAP, after the depletion of available soil-P. No-P or low-P treatment of NM plants resulted in substantially less growth and photosynthetic surface area, lower tissue-P concentrations, and an altered plant morphology when compared with high-P treatment of NM plants. Furthermore, inadequate P nutrition extensively inhibited the uptake of mineral nutrients other than P (Fig. 8), probably due to a reduced capacity to intercept and absorb ions. In this regard, an average 27% lower root dry mass for no-P NM plants than for high-P NM plants between 28 and 84 DAP (Fig. 3) indicated a reduction in root surface absorptive area by P stress (Newman and Andrews, 1973). Changes to the ion-selectivity of roots and a diminished metabolic capacity for ion uptake have also been implicated in lower mineral nutrient accumulation by P deficient plants (Baas et al., 1989; Lee, 1982; McArthur and Knowles, 1993). Although shoot-N concentration was not altered by P nutrition, N assimilation appeared less efficient in P deficient plants, as the level of insoluble-N and also reduced-N total-N^{-1} were lower for no-P and low-P plants than for high-P plants (Table IV). In this respect, leaf nitrate reductase activity has been demonstrated to be less for low-P than for high-P potato plants (McArthur and Knowles, 1993). These responses of potato plants to low P nutrition are similar to those reported for other plants grown with inadequate P supply (Fredeen et al., 1989; Lambert et al., 1979) or exposed to short-term P deprivation (Cogliatti and Clarkson, 1983; Goldstein et al.,

1988; Rufty et al., 1990). Such responses are generally attributed to P deficiency stress.

Comparison of root and shoot growth of plants grown with different levels of P nutrition demonstrated that roots were less affected by P deficiency than shoots (Fig. 3 and Table II). Since P tends to be immobile in soils, continued root elongation at the expense of shoot growth is a primary plant strategy for maintaining P acquisition under deficiency conditions (Clarkson, 1985). Moreover, roots of P-stressed plants were more efficient than high-P plants in acquiring available P from the soil, as demonstrated by the higher values of P-accumulated/P-available and RSAR for no-P plants than for high-P plants (Fig. 5 and inset). These findings are consistent with the view that P starvation enhances the affinity and transport capacity of potato roots for P. Unlike the rapid induction of a high-affinity system (within 3 d) by complete P deprivation as observed by Cogliatti and Clarkson (1983), depletion of soil P (28 to 56 DAP) was required in our study before such physiological changes were evident. Similar responses to P deficiency by Russet Burbank potato have been previously reported and were associated with enhanced root acid phosphatase and ATPase activities (McArthur and Knowles, 1993). Collectively, these responses to P deficiency appear to constitute part of a "phosphate starvation rescue system" in higher plants (Goldstein et al., 1988).

Growth and development of P deficient plants were markedly improved by inoculation with any one of the three species of VAM fungi, although the plant response to *G. dimorphicum* was generally less than that to *G. intraradices* or *G. mosseae*. Such differences between VAM species appear to relate to their ability to colonize roots (Abbott and Robson, 1982) and, although not analyzed here, to their production of external hyphae required for P acquisition (Sanders et al., 1977). In this regard, VAM roots were more efficient at P uptake than NM roots (Fig. 5 and insets), as observed by others (Sanders et al., 1977; Cress et al., 1979; Baas et al., 1989). Although the VAM symbiosis produced marginally higher shoot-P concentrations in plants by 28 DAP, significant changes to plant P accumulation occurred sometime after 28 d (Fig. 5, inset). It is of interest that, in spite of the inhibitory influence that high abiotic P supply to plants had on fungal growth (Fig. 1, inset), the fungal symbionts were clearly successful in maintaining the exchange of P and carbohydrates with the host, as evidenced by the high level of root infection and by the enhanced P content of high-P VAM plants at 84 DAP (Fig. 4). The fact that the growth response of high-P plants to VAM infection was negligible, in spite of a VAM-enhancement of P uptake, suggests that any increase in carbon assimilation resulting from improved plant P-nutrition was offset

by symbiont consumption, as was suggested by Baas et al. (1989) and as indicated by a diminished ULR for high-P VAM plants (Table III).

Comparisons of the polygonal diagrams in Figure 3 and Figure 8 show that improvements to growth and development of P deficient plants by the VAM symbiosis were at least partially due to alleviation of the P deficiency-induced restriction of mineral nutrient uptake and transport to shoots. One of the most immediate plant benefits of the VAM symbiosis was a stimulation of leaf growth and expansion, which was evident by 56 DAP and associated with a significantly greater total accumulation of P, N, K, and Mg for plants grown with each level of abiotic P supply. Even preceding these effects, the concentrations of P, N, and Mg in shoots were already significantly higher by 28 DAP in VAM plants than in NM plants. By such enhancement in shoots of not only P, but also other elements which promote axillary bud and leaf growth (Woolley and Wareing, 1972; Radin and Boyer, 1982), the VAM symbiont appeared to stimulate the development of plant photosynthetic surface area. Conversely, tuber growth, which might compete with the VAM symbiont for photoassimilates, was negatively influenced by VAM (Table II). This could be specifically ascribed to enhanced N accumulation and subsequently higher tissue-N levels for VAM plants (Fig. 6 and 8), which are known to delay tuber initiation and growth (Krauss, 1985). The influence of N content on tuberization appeared to be temporary, as shoot-N concentrations dropped later in the study. In fact, tuber yield is increased by a VAM symbiosis under tuber-inducing conditions, as P uptake is significantly greater for VAM plants than NM plants during tuber-bulking when the sink activity of tubers for P may deplete shoot-P and thus limit photosynthesis (Nelson et al., 1947). In this regard, we have documented greater tuber yields for VAM plants than for NM plants when grown with suboptimal P nutrition under field or controlled environment conditions (McArthur and Knowles, 1991; McArthur and Knowles, 1993).

A rather unusual response by potato plants to the VAM symbiosis was the decrease in shoot-Zn concentrations (Fig. 7B). While the shoot-Zn concentration response to increasing abiotic P-supply was consistent with that previously reported (Lambert et al., 1979; Loneragan et al., 1979), our result of a significantly lower shoot-Zn concentration in VAM-infected plants is contrary to most reports (Lambert et al., 1979; Marschner, 1986; Kucey and Janzen, 1987). Since concentrations of shoot-Zn in our study were high relative to other studies in which Zn levels were noticeably deficient, transfer of Zn by VAM fungi to plants in our study was probably not of significant benefit relative to the transfer of P. Therefore, it seems possible that the greater accumulation of Zn under P deficient conditions in VAM plants than in NM plants

(Fig. 8) reflects improved uptake of Zn by roots after alleviation of P deficiency stress, similar to that for for N, K, Mg, and Fe. Finally, the lower shoot-Zn concentrations of VAM plants than for NM plants may be attributable to P x Zn interactions involving intraradical hyphae and root cells, which have yet to be characterized (Robson and Pitman, 1983).

In summary, our results provide the first detailed characterization of the influence of P deficiency stress and VAM symbiosis on mineral physiology and early growth of potato, and illustrate the complexity that differential P nutrition has on plant processes. The prohibitive influence of P deficiency stress on mineral nutrient acquisition of potato underscores the importance of P nutrition to maintaining optimal nutrient-utilization efficiency in general. Species of VAM fungi differed in their ability to benefit plant P nutrition under our growth conditions; however, further investigation is recommended to determine why this was so and whether similar results can be achieved under field conditions.

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Chapter III

Influence of Vesicular-Arbuscular Mycorrhizal Fungi on the Response of Potato to P Deficiency[2]

Introduction

Second only to N, P is the mineral nutrient for which availability is most likely limiting to plant growth. The concentration of P in the soil solution is in the micromolar range, and moreover, this element exhibits slow diffusion in soils. Hence, depletion of P in the root zone commonly limits further uptake of P by existing roots and, potentially, by the plant as a whole (Bieleski and Ferguson, 1983; Clarkson, 1985). The effects of P starvation on plant growth and development are pleiotropic, and include the restriction of cell division and expansion, inhibition of photosynthesis and respiration, and impairment of root uptake and transport of other nutrients, such as NO_3 (Bieleski and Ferguson, 1983; Baas et al., 1989; Fredeen et al., 1989; Rufty et al., 1989). For the potato (*Solanum tuberosum* L.), a crop of world-wide agronomic importance, these effects result in substantial reductions in yield (Harris, 1978; Pursgove and Sanders, 1981; MacKay et al., 1988).

Potato plants have been characterized as relatively inefficient at acquiring soil P (Pursgove and Sanders, 1981), but are capable of morphological and physiological adaptations during the development of P deficiency that may substantially improve P acquisition (Cogliatti and Clarkson, 1983). Such adaptations include changes in P and dry matter partitioning that favor growth of roots over shoots, and the induction of a high-affinity P uptake and transport system in roots. These responses to P deficiency are not exclusive to potato, and, while not demonstrated specifically for potato, other mechanisms by which plants cope with inadequate P supply have been described (Clarkson, 1985). For example, excretion of Apases[2] by roots and enhanced acidification of the rhizosphere by plasma membrane-associated ATPases are thought to speed dissociation of P from P-containing compounds, thus improving uptake from various soil P pools (Clarkson, 1985; Leonard, 1985; Goldstein et al., 1988). Promotion of root extension and root hair formation also contribute significantly to more efficient P uptake (Barber and Silberbush, 1984; Clarkson, 1985).

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An alternate plant strategy for coping with P deficiency is the establishment of a mycorrhizal symbiosis with an appropriate fungus. External hyphae of VAM roots more rapidly exploit a given volume of soil for available P than roots of NM plants, and thus speed the acquisition of soil P for an infected root (Harley and Smith, 1983). For plants such as potato, which have a low root density (Pursglove and Sanders, 1981) and high growth potential, the VAM symbiosis may be of particular significance in coping with P deficiency stress in natural ecosystems. This also appears true for the commercial production of potato, as significant yield increases due to VAM fungi have been recorded (Black and Tinker, 1977; McArthur and Knowles, 1991).

The compatibility of plants to VAM fungi is enhanced under conditions of P deficiency (Schwab et al., 1991) in a manner similar to those physiological processes implicated in the 'rescue' of plants from P starvation (Goldstein et al., 1988). Factors that may be involved in plant compatibility to VAM fungi have recently been reviewed (Koide and Schreiner, 1992), and may be nutritional, based on either fungal symbiont recognition by plants, or by avoidance of a defense response by the fungal symbiont (Anderson, 1988; Schwab et al., 1991; McArthur and Knowles, 1992). While the molecular mechanisms responsible for plant compatibility to VAM fungi have not been characterized, the plant's susceptibility to VAM infection appears somehow linked to P starvation-induced physiology of plants (McArthur and Knowles, 1992).

The intent of this study was to characterize morphological and biochemical aspects of P deficiency in potato as affected by VAM fungi, specifically *Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe. The extent to which VAM infection altered physiological processes that were also affected in potato by P deficiency stress was central to our study. Since increased susceptibility to VAM fungi was demonstrated to be one response to P deficiency, we suggest that, once identified, factors influencing susceptibility to VAM infection also be considered as components of P starvation-induced metabolism.

MATERIALS AND METHODS

Inocula

Pure pot culture of *G. fasciculatum* originated from single-spore culture of chlamydospores isolated from roots of strawberry grown in Edmonton (53° 30' N Lat. 113° 30' W Long.). Differences between isolates were not evident and, moreover, chlamydospores produced in pot culture were of one type, which consistently matched the description of Walker and Koske (1987). Inocula for NM and VAM (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) treatments consisted of

soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved sand:soil (3:1 v/v) medium for 90 d. Plants were grown in 2-L pots and were fertilized weekly with 50 mL of a modified (minus P) Hoagland's solution. Roots of NM clover plants were uninfected, while those of VAM plants were 85% infected at 90 DAP and had many vesicles. Few mature chlamydospores were present on either the surface of roots or in the soil of the VAM inoculum. To quantify infection for plants at 40, 54 and 63 DAP (see below), roots were cut to 1-cm lengths and stored in FAA solution (5% formalin, 5% acetic acid, 45% ethanol) until they were cleared and stained (Phillips and Hayman, 1970). Percent infection was assessed by the gridline intersect method (Giovanetti and Mosse, 1980) with 100 counts per slide, and five (clover) or three (potato [*Solanum tuberosum* L. cv Russet Burbank]) slides per plant.

Plant Growth Conditions and Experimental Design

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface-sterilized with 1.0% (v/v) sodium hypochlorate (3 min) and rinsed thoroughly with distilled H_2O. Single-eye seedpieces (5.0 g) were cut from the mid-region of the tubers and rinsed. After air-drying for 1 h, seedpieces were planted in vermiculite and sprouted in the dark for 10 d at 27°C (95% RH).

Single-sprout seedpieces were blocked for sprout-length prior to transplanting onto NM or VAM soil inoculum (100 g fresh weight pot^{-1}) in 20-cm diameter pots (1 seedpiece pot^{-1}). Each pot contained 3.4 kg (air-dry) of an autoclaved soil medium consisting of 6:1 v/v sand:soil (5 $\mu g\ g^{-1}$soil $NaHCO_3$-extractable P). An equal mix of fine and coarse sand was used in the medium, and the soil was a silty clay loam (Malmo Typic haploboroll), with a cation exchange capacity of 0.5 (millimhos cm^{-1}). A 2:1 (v/v) water extract of this soil had a pH of 6.2, no detectable P, 33 µg total-N, 40 µg Ca, 11 µg Mg, and 5 µg K (g^{-1} soil). Pots were placed in a growth chamber set at 25/20°C (day/night) with a 16-h photoperiod. A PPFD of 480 $\mu mol\ m^{-2}\ s^{-1}$ was provided by fluorescent and incandescent lights maintained at 25 cm from shoot tips. In the first week, each pot received 50 mL of nutrient solution three times [(mM): 40, KNO_3; 20, $Ca(NO_3)_2$; 20, $MgSO_4$; µM: 185, H_3BO_3; 36.5, $MnCl_3$; 0.3, $ZnSO_4$; 1.3 $CuSO_4$; 0.065 H_2MoO_4; 2 mg/L FeEDDHA (pH 6.0)], then 100 mL with added P (0.5 or 2.5 mM NaH_2PO_4) was given three times per week thereafter (24 applications total). Thus, treatments consisted of two levels of P (0.5, 2.5 mM), and two inocula (NM or VAM) arranged factorially in a randomized complete block design. Growth data were collected for plants harvested for physiological studies and were grouped into harvest dates of 40 and 54 DAP (four blocks each). Plants harvested at 63 DAP (four blocks)

were used for growth data also and from these, LAR (LA/W), S_W/R_W,(T_W not included with S_W), and T_W/W at 63 DAP were calculated. Shoots, tubers, and roots were lyophilized for P, carbohydrate, and N analyses. P-accumulation/P-available = (cumulative total of shoot P plus tuber P at each harvest date minus initial plant P at transplanting)/(cumulative total of P supplied at each harvest date, including total $NaHCO_3$-extractable soil P pot^{-1}).

Nitrate Reductase Activity

Sixty uniform leaf discs for a total of 17 cm^2 $plant^{-1}$ were taken from each treatment and each of three blocks at 42 and 56 DAP. Samples were incubated in darkness in 10 mL of a 1 mM phosphate buffer (pH 7.5) containing 0.3 M KNO_3 at 25°C for 100 min. Nitrite concentrations were determined at 20-min intervals by the method of Sanderson and Cocking (1964) and nitrate reductase activity was expressed as µmol NO_2^- g^{-1} dry weight h^{-1}.

Root Respiration

Root respiration was measured at 33, 38, 43, 45, 57, and 59 DAP (average 46). Roots of intact plants were gently washed in running water, rinsed again in cold deionized-distilled H_2O, and stored briefly (max 30 min) in cold deionized-distilled H_2O (4°C). Roots were then blotted dry with filter paper, and fine roots from the mid-region of each root system were cut into 1-cm lengths. Excised roots were placed in triplicate 15-mL disposable test tubes (1 g fresh weight $tube^{-1}$) containing 2 mL of 50 mM Mes (Tris) buffer (pH 6.5). Tubes were capped with serum stoppers and incubated at 23°C for 5 h. Production of CO_2 by excised roots was measured at 2.5-h intervals by injecting a 1-mL gas sample from each tube into a Hewlett-Packard, model 5890A gas chromatograph equipped with a thermal conductivity detector. The 2.4 m stainless steel column (3.2 mm o.d.) was packed with 80/100 mesh HayeSep T (Hewlett-Packard). The He flow rate was 30 mL/min and the column was isothermal at 100°C. The 1-mL gas samples were replaced with an equal volume of air at each sampling. Excised roots were later lyophilized and respiration was expressed as µmol CO_2 g^{-1} dry weight h^{-1}.

Microsomal ATPase and Apase Assays

Root microsomal ATPase activity was measured at 38, 47, and 53 DAP. All operations were carried out at 4°C. Roots from the mid-section of the root-system were cut into 2-mm pieces and ground in a mortar and pestle for 3 min with homogenization buffer (7 g fresh weight to 21 mL) [250 mM sucrose, 2 mM EDTA, 2 mM DTT, 0.05%

BSA (w/v) and 0.5 mM PMSF in 50 mM Tris (Mes), pH 7.4]. Homogenate was filtered through miracloth, and the filtrate was centrifuged at 15,000g for 25 min. The supernatant was then centrifuged at 105,000 g for 35 min. The pellet was gently resuspended with a glass homogenizer in 21 mL of resuspension buffer (250 mM sucrose, 2 mM DTT, 10 mM Tris (Mes) pH 7.4) and re-centrifuged at 105,000 g (12). The pellet was then resuspended with 4 mL of resuspension buffer and stored overnight at 4°C.

Root microsomal ATPase activity was assayed by determination of P from ATP hydrolysis (Serrano et al., 1976) after 30 min of incubation at 37°C. In a total volume of 0.5 mL, the reaction was started by addition of 25 µL of membrane suspension (10-25 µg protein) to 50 mM Mes (Tris) buffer (pH 6.5) containing 2.5 mM ATP (Vanadium-free, Sigma), 2.5 mM $MgSO_4$, and 1 mM molybdate. ATPase activity was terminated by addition of 25 µL of 24% (w/v) TCA, followed by 2 mL of 0.7% (w/v) $(NH_4)_6MoO_7{\cdot}O_{24}{\cdot}4H_2O$ in 0.72 N H_2SO_4. The specific activity of ATPase (µmol P mg^{-1} protein h^{-1}) was characterized over a pH range of 5.5 to 7.5 and showed a pH optimum of 6.0 (specific activity = -107 + 45.5(pH) - $3.8(pH)^2$, r = 0.99).

In preliminary studies on ATPase activity, the inhibitors, nitrate (50 mM KNO_3), molybdate (1 mM $Na_2MoO_4{\cdot}2H_2O$) or azide (1 mM NaN_3), were separately tested to estimate the presence of vacuolar ATPases, nonspecific Apases, or mitochondrial ATPases, respectively. ATP hydrolysis was inhibited ($P < 0.01$) 14% by molybdate, but not by azide or nitrate. This concentration of molybdate strongly inhibits Apase activity, but only slightly decreases ATPase activity (Gallagher and Leonard, 1982). Hence, 1 mM molybdate was included in all further ATPase assays to inhibit Apase activity. Vanadate sensitivity (0.1 mM $NaVO_4$ or V_2O_5) was used to demonstrate the presence of plasma membrane ATPases in microsomes. After accounting for possible interference in the P assay by vanadate (an absorbance shift of -0.04), vanadate inhibition of the specific activity of ATPase was determined to be 24% ($P < 0.01$).

At 47 and 53 DAP, root microsomal Apase activity was assayed in a 50 mM Mes (Tris) reaction buffer (pH 5.5) containing 2.5 mM *p*NPP and 2.5 mM $MgSO_4$ (Gallagher and Leonard, 1982). Nitrophenol was used as a standard. To start the reaction, 25 µL of the microsomal membrane preparation was added to 475 µL of reaction buffer and then incubated for 30 min at 37°C. The reaction was stopped with 1 mL of 1 N NaOH and the A_{410} was determined. Apase activity in the 105,000 g supernatant was similarly assayed at 47 DAP.

Soluble-N, Soluble Carbohydrate, and Total P Assays

For determination of plant N-metabolites, P, and carbohydrate content, lyophilized plant material from 40, 54, and 63 DAP was ground through a Wiley mill (40 mesh). Fifty mg was extracted (4°C) for 2 min (mortar and pestle) in 5 mL of 50 mM Hepes buffer (pH 7.4). Homogenate was centrifuged (1640 g, 4°C) for 30 min and free amino-N, soluble protein-N, and NO_3-N were determined on 100, 200, and 50 µL of cold supernatant, respectively. Soluble protein-N was measured by a modified Lowry method (Bensadoun and Weinstein, 1976) with BSA (15.6% N) as the standard. Ninhydrin was used to assay free amino-N (Rosen, 1956) with leucine (10.7% N) as the standard. Nitrate-N was determined by the methods of Cataldo et al. (1975) with KNO_3 as the standard. Reducing sugars were assayed colorimetrically by the method of Somogyi (1952) with a 100-µL aliquot of extract and glucose as the standard. Total soluble carbohydrates were determined on a 50-µL aliquot of supernatant with the phenol-sulfuric acid reagent (Dubois et al., 1956) and glucose as the standard. Plant material was dry-ashed and total P determined by the method of Serrano et al. (1976).

Microscopy

For dark field microscopy, 1-cm root lengths were stained with lactophenol cotton blue. Localization of cellular dehydrogenase activity in VAM roots was determined by a 20-h incubation in 50 mM Hepes (pH 7.4) containing 2 mg MTT/mL (An and Hendrix, 1988).

For electron microscopy, washed roots were cut to 3-mm lengths in 100 mM phosphate buffer (pH 6.8), transferred to phosphate buffer containing 2.5% glutaraldehyde and incubated for 2 h at 23°C. Roots were then rinsed three times with phosphate buffer. For cryostage scanning EM, roots were frozen in liquid N_2 and fractured with an Emitech K1250 Scanning EM Cryosystem. Water was sublimated from the fractured surface of frozen roots for 30 min before examination. Alternatively, roots were incubated in phosphate buffer containing 2% OsO_4 for 2 h and rinsed three times with phosphate buffer. Roots were then plunged into liquid N_2, fractured with a pre-cooled scalpel, and lyophilized overnight. Roots were mounted on double-sided sticky tape on stubs, gold-sputter-coated, and viewed with a Cambridge Stereoscan 150 scanning electron microscope. Glutaraldehyde-osmium-fixed roots were also embedded in Spurrs resin, thin sectioned, and stained with uranyl acetate followed by lead citrate for viewing with transmission EM. For freeze-fracture replica processing, glutaraldehyde-treated roots were incubated in phosphate buffer (pH 7.4) containing 30% glycerol for 30 min (Hudson et al., 1981). Root pieces were oriented

longitudinally onto gold discs, plunged into liquid-phase Freon-22, stored in liquid N_2, and then transferred into a pre-cooled Balzers BAE 080 apparatus (Balzers Instruments, Lichtenstein). Roots were fractured at -110°C and shadow-coated with platinum and carbon. The replicas were cleaned with chromic acid and washed with distilled H_2O. Replicas and thin sections were mounted on formvar-coated grids and examined with either a Siemens Ehniskop 102 or a Hitachi H-7000 electron microscope.

Statistical Analyses

Growth and physiological data were subjected to ANOVA and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Percent-infection data were arcsin transformed to achieve homogeneity of variance prior to ANOVA. Based on the results of ANOVA, regression analysis was used to derive polynomial models for describing various relationships. In this study, $Time_{deviation}$ indicates a significant residual variance that is not explained by a linear model, and with no further trend analysis possible.

RESULTS

Analysis of Plant Growth

Plant growth was clearly limited by low abiotic P supply (0.5 mM), as dry matter accumulation in shoots and tubers of low-P NM plants at 40 DAP was 35% and 71% lower, respectively, than that from high-P (2.5 mM) NM plants (Fig. 1A and 1B). By 63 DAP, roots, shoots, and tubers of low-P NM plants had 34% (Fig. 1C), 52%, and 73% less dry matter, respectively, than those from plants grown with high-P. Inoculation of plants with *G. fasciculatum* significantly enhanced dry matter production in shoots and roots of low-P plants relative to low-P NM plants. In spite of this growth enhancement, the total dry weight of high-P NM plants at 63 DAP was still two-fold greater than that of VAM plants grown with low abiotic P supply. Under high-P conditions, VAM infection marginally increased root dry weight but did not significantly influence the final whole-plant dry weight.

The most appreciable influence of P nutrition on plant morphology was observed in leaf development. At 63 DAP, the total leaf area of low-P NM plants was less than half that of high-P NM plants, due to a combination of a 35% reduction in area per leaf and 39% fewer leaves (Table I). Total leaf area at 63 DAP was greater at both abiotic P levels for VAM plants than for NM plants (Table I). Under low-P conditions, a higher leaf area for VAM plants could be ascribed to improved growth due to the symbiosis (Fig.

Figure III-1. Main effects of time and abiotic P supply on dry matter accumulation of shoots (A) and tubers (B) for potato plants inoculated with NM (○, □) or VAM (*G. fasciculatum*) (●, ■) clover inoculum. Plants were grown with either 0.5 mM P (○, ●) or 2.5 mM P (□, ■). Treatment effects on the dry weight of roots at 63 DAP (C) are also shown. For dry matter accumulation of shoots, F-values for the main effects of $Time_{linear}$, VAM, [P], and interactions $Time_{linear}$ x [P] and VAM x [P] were significant at the 0.01, 0.01, 0.01, 0.01, and 0.05 levels, respectively. For dry matter accumulation of tubers, F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, [P], and the interaction $Time_{linear}$ x [P] were significant at the 0.01, 0.05, 0.01, and 0.01 levels, respectively. For root dry weights, F-values for the main effects of VAM and [P] were significant at the 0.05 and 0.01 levels, respectively.

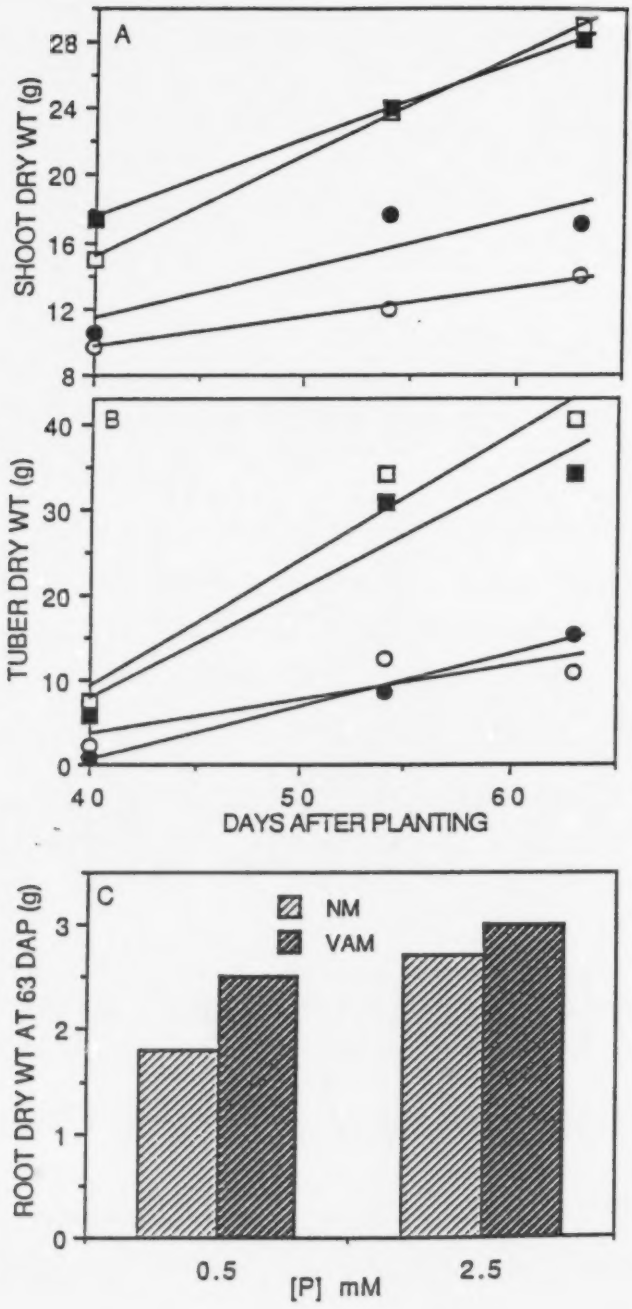

A
SHOOT DRY WT (g)
28
24
20
16
12
8
B
TUBER DRY WT (g)
40
30
20
10
0
40
50
60
DAYS AFTER PLANTING
C
ROOT DRY WT AT 63 DAP (g)
NM
VAM
3
2
1
0
0.5
2.5
[P] mM

Table I. *Effect of a Vesicular-Arbuscular Mycorrhizal (VAM) Fungus and P Supply on Morphology of Potato Plants*

Plants were inoculated with nonmycorrhizal or VAM (*Glomus fasciculatum*) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 mM or 2.5 mM P. Plants were harvested at 63 DAP.

Treatment		Leaf Number	Leaf Area	Total Leaf Area	Leaf Area Ratio	Shoot Root^{-1} Wt Ratio	Tuber[a] Wt Ratio
[P]	VAM		$dm^2\ leaf^{-1}$	$dm^2\ plant^{-1}$	$dm^2 g^{-1}\ dry\ wt$		
0.5	-	100	0.17	15.9	0.60	8.2	0.40
	+	108	0.24	24.5	0.71	6.9	0.43
2.5	-	165	0.26	39.8	0.56	10.8	0.56
	+	153	0.29	44.3	0.68	9.4	0.52
[P][b]		0.01[c]	0.05	0.01	ns	0.01	0.05
VAM		ns	0.10	0.01	0.05	0.05	ns
[P] x VAM		ns	ns	ns	ns	ns	ns

[a], Tuber Wt Ratio, tuber:plant dry wt ratio.

[b] Sources of variation. [c] Significance levels for indicated sources of variation (ns = not significant).

1A) and hence, a greater amount of photoassimilates for shoot development. However, no such improvement in growth was evident for VAM plants grown with high abiotic P supply. Since leaf number did not differ between NM and VAM plants, and VAM plants had a significantly greater area per leaf and LAR than NM plants (Table I), a VAM influence on leaf expansion was mostly responsible for the greater total leaf area.

A major influence of P nutrition on the concentrations of plant soluble carbohydrates during the 63-d growth period was apparent by the relative proportion of non reducing sugars to reducing sugars (Table II). The ratio of non reducing to reducing sugars was higher for shoots or roots of low-P plants than for high-P plants. Since shoot and root growth were restricted by low abiotic P supply (Fig. 1), the higher proportion of non reducing sugars in low-P plants likely reflects a reduced capacity to utilize carbohydrates for growth and hence, greater vacuolar storage of non reducing sugars such as sucrose (Mares et al., 1985). In shoots, differences between the levels of reducing sugars and non reducing sugars for low-P and high-P plants offset each other. In contrast, the lower root concentration of reducing sugars for low-P plants was only partially offset by the higher level of non reducing sugars. Thus, total soluble carbohydrates were significantly lower in roots of low-P plants than in roots of high-P plants. Such altered root carbohydrate levels due to P deficiency have been reported for other plant species and are often associated with enhanced exudation of reducing sugars into the rhizosphere (Schwab et al., 1991). Such exudates may enhance VAM infection but were not measured in our study. No influence of VAM infection on root carbohydrate levels was evident, although the concentration of shoot carbohydrates for VAM plants was marginally lower than that for NM plants (Table II).

The effects of abiotic P supply and VAM infection on soluble-N pools were also characterized for potato plants (Table III). Over the harvest interval, shoot concentrations of nitrate-N, free amino acid-N and soluble protein-N were significantly higher for low-P than for high-P plants; a response which was most likely due to the relatively slow growth of shoots (Fig. 1A). The higher concentration of nitrate-N in shoots of low-P plants was associated with lower leaf nitrate reductase activity relative to that in leaves of high-P plants (Table III). Since the ratio of soluble reduced-N to total soluble-N was not altered by P nutrition, the results indicate that N-assimilation was perhaps slower but not impaired. VAM infection had no effect on the concentrations of soluble shoot N-metabolites, although nitrate reductase activity for high-P VAM plants was 20% greater than comparable NM plants. A similar enhancement of nitrate reductase activity was reported in roots and leaves of VAM-infected clover, and was

Table II. *Effect of a Vesicular-Arbuscular Mycorrhizal (VAM) Fungus and P Supply on soluble carbohydrates of Potato Plants*

Plants were inoculated with nonmycorrhizal or VAM (*Glomus fasciculatum*) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 mM or 2.5 mM P. Results from plants harvested at 40, 54, and 63 DAP were pooled.

Treatment		Shoot Soluble Carbohydrates				Root Soluble Carbohydrates			
		Reducing Sugars	Non reducing Sugars	Total Sugars	NRS/RS[a]	Reducing Sugars	Non reducing Sugars	Total Sugars	NRS/RS
[P]	VAM	*mg g^{-1} dry wt*				*mg g^{-1} dry wt*			
0.5	-	24.1	44.6	68.7	2.0	8.7	27.6	36.3	3.5
	+	22.8	42.3	65.1	1.9	9.7	26.2	35.9	3.0
2.5	-	31.6	35.4	67.0	1.1	14.9	25.3	40.2	2.3
	+	28.5	35.2	63.7	1.5	15.8	23.8	39.6	1.8
[P][b]		0.01[c]	0.01	ns	0.01	0.01	0.05	0.05	0.01
VAM		0.10	ns	0.05	ns	ns	ns	ns	ns
[P] x VAM		ns	ns	ns	ns	ns	ns	ns	ns

[a], NRS/RS, non reducing sugars:reducing sugars dry wt ratio.
[b] Sources of variation. [c] Significance levels for indicated sources of variation (ns = not significant).

Table III. *Effect of a Vesicular-Arbuscular Mycorrhizal (VAM) Fungus and P Supply on soluble N-Pools and in vivo Leaf Nitrate Reductase activity of Potato Plants*

Plants were inoculated with nonmycorrhizal or VAM (*Glomus fasciculatum*) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 mM or 2.5 mM P. Results for plants harvested at 40, 54, and 63 DAP were pooled.

Treatment		Soluble Shoot-N					Soluble Root-N			
		NO_3^-	Amino	Protein	Reduced Total^{-1}	Leaf NRA[a]	NO_3^-	Amino	Protein	Reduced Total^{-1}
[P]	VAM	*mg g^{-1} dry wt*				*µmol g^{-1} dry wt h^{-1}*	*mg g^{-1} dry wt*			
0.5	-	9.0	2.2	12.1	0.61	6.3	10.0	1.4	6.2	0.42
	+	9.5	2.4	12.9	0.62	6.4	6.0	1.7	7.7	0.60
2.5	-	8.1	1.9	10.4	0.60	7.4	7.4	1.4	6.6	0.50
	+	7.4	1.9	11.0	0.63	8.9	5.0	1.7	8.3	0.66
[P][b]		0.01[c]	0.01	0.01	ns	0.01	0.01	ns	ns	0.05
VAM		ns	ns	ns	ns	0.01	0.01	0.01	0.01	0.01
[P] x VAM		ns	ns	ns	ns	0.01	0.10	ns	ns	ns

[a], NRA, nitrate reductase activity, µmol NO_2^- g^{-1} dry wt h^{-1} at 42 and 56 DAP.
[b] Sources of variation. [c] Significance levels for indicated sources of variation (ns = not significant).

attributed to improved P nutrition provided by the VAM symbiosis (Oliver et al., 1983).

In contrast to shoot N-pools, P nutrition had no significant effect on either free amino acid-N or soluble protein-N concentration in roots (Table III). However, the nitrate-N concentration was 35% greater in low-P than in high-P roots and hence, the ratio of soluble reduced-N to total soluble-N was significantly lower for roots of low-P plants. The high root nitrate-N level in NM plants indicates that root N-assimilation or nitrate transport to shoots was unable to match uptake, resulting in more storage of nitrate in roots of low-P plants. Changes to root soluble-N pools in response to VAM infection were more obvious and were characterized by significantly lower nitrate-N and higher levels of reduced-N (mg g^{-1} dry weight). This shift in soluble-N pools resulted in a 37% higher ratio of reduced-N to total soluble-N in roots of VAM plants than in NM plants.

Continuous plant growth with limited P supply resulted in a gradual decline in tissue P concentrations (Fig. 2). In spite of the slower growth of low-P plants, by 40 DAP the P concentrations of roots, shoots, and tubers were already 36%, 28%, and 19% lower, respectively, than those of high-P NM plants (Fig. 2). Over the remaining harvest interval, the relative decline in tissue P was less pronounced in low-P plants than in high-P plants, reflecting the almost negligible growth of low-P plants between 54 and 63 DAP. Hence, by 63 DAP, the P concentrations of roots and shoots of high-P NM plants approached those values maintained in low-P NM plants. The absence of such a trend for tuber P concentration (Fig. 2B), despite substantial tuber dry mass accumulation up to 63 DAP for all treatments (Fig. 1B), suggests that the sink strength of tubers for P was high during this period.

Although plant growth was only moderately stimulated by the VAM symbiosis, tissue P concentrations of VAM plants were significantly greater than those of NM plants throughout the harvest interval at either abiotic P level (Fig. 2). Over the 23-d harvest period, P was 13%, 12%, and 54% more concentrated in shoot, tuber, and root tissues, respectively, of VAM plants than in NM plants. For low-P plants, VAM infection also increased total P accumulation at 63 DAP in shoots, tubers, and roots by 31%, 53%, and 112%, respectively, (Table IV). For high-P plants, however, no net benefit of VAM infection on total P content was evident, as a marginal increase in shoot P was offset by a decrease in tuber P. Although roots of high-P VAM plants contained 79% more P than those of high-P NM plants, this increment to whole-plant P content (2.3 mg P) was almost negligible.

Figure III-2. Time course of P concentrations of shoots (A), tubers (B), and roots (C) of potato plants inoculated with NM (○, □) or VAM (●, ■) (*G. fasciculatum*) clover inoculum and grown with 0.5 mM (○, ●) or 2.5 mM P (□, ■). For shoot P, F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, VAM, [P], and the interaction $Time_{linear}$ x [P] were significant at the 0.01, 0.01, 0.01, 0.01, and 0.05 levels, respectively. For tuber P, F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, VAM, and [P] were significant at the 0.01, 0.01, 0.05, and 0.01 levels, respectively. For root P, F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, VAM, [P], and the interaction $Time_{linear}$ x [P] were significant at the 0.01, 0.10, 0.01, 0.01, and 0.10 levels, respectively.

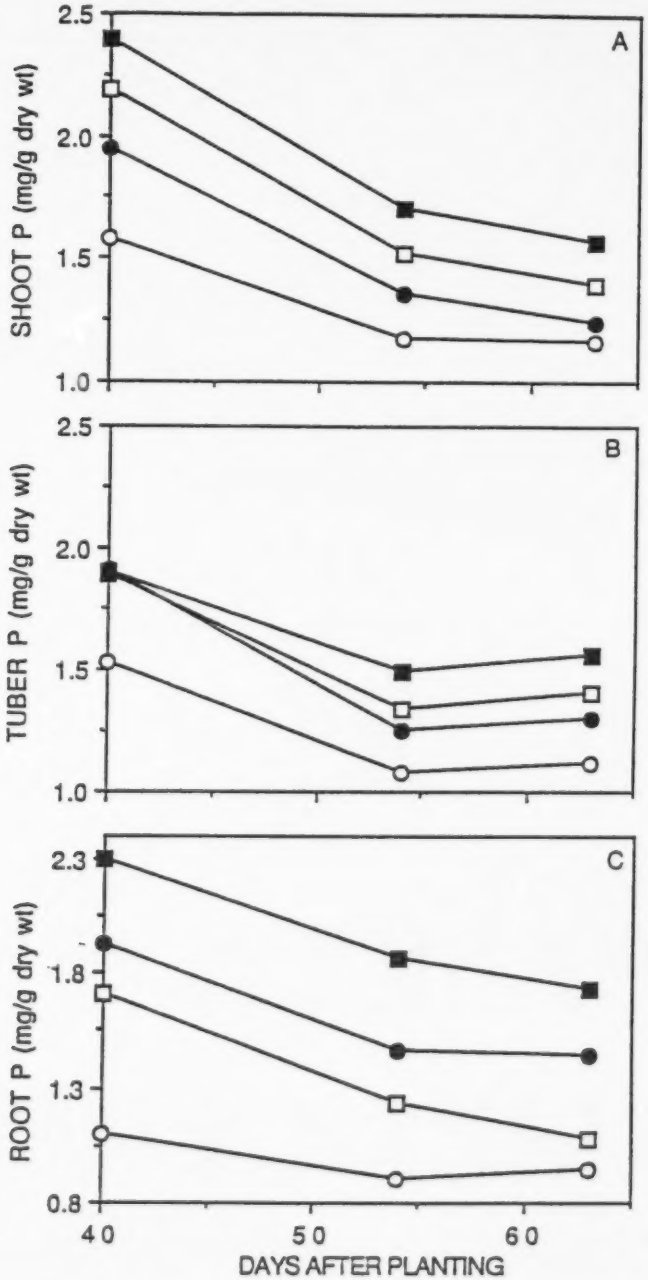

A
B
C
SHOOT P (mg/g dry wt)
TUBER P (mg/g dry wt)
ROOT P (mg/g dry wt)
2.5
2.0
1.5
1.0
2.3
1.8
1.3
0.8
40
50
60
DAYS AFTER PLANTING

Table IV. *Effect of a Vesicular-Arbuscular Mycorrhizal (VAM) Fungus and P Supply on P Accumulation of Potato Plants*

Plants were inoculated with nonmycorrhizal or VAM (*Glomus fasciculatum*) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 mM or 2.5 mM P.

Treatment		P Accumulated/P available[a]			Shoot P	Tuber P	Root P	Plant P
[P]	VAM	%			mg plant^{-1} at 63 DAP			
		40 DAP	*54 DAP*	*63 DAP*				
0.5	-	55	64	57	16.3	11.8	1.7	29.7
	+	65	77	81	21.4	18.1	3.6	43.0
2.5	-	38	48	49	40.2	56.9	2.9	100.0
	+	43	50	49	44.3	52.5	5.2	102.0
Time[a]			L, D[b]		-	-	-	-
[P][b]			0.01		0.01	0.01	0.01	0.01
VAM			0.01		0.10	ns	0.01	0.01
[P] x VAM			0.01		ns	0.10	ns	0.01

[a], Based on cumulative total of shoot P plus tuber P at each harvest date minus initial plant P at transplanting *versus* cumulative total of P supplied at each harvest date, including total $NaHCO_3$-extractable soil P pot^{-1}.

[b] Sources of variation. [c] Significance levels for indicated sources of variation (L = linear trend; D = deviation from linear trend; ns = not significant).

The increase in P nutrition of host plants provided by the VAM symbiont did not totally compensate for low abiotic P supply, and low-P VAM plants had 57% less P at 63 DAP than NM plants with high-P (Table IV). However, the VAM symbiosis definitely improved plant P nutrition over that of low-P NM plants, which had 70% less P than high-P NM plants. In spite of their relatively low P content, a much higher proportion of available P was recovered by low-P plants than by high-P plants (Table IV). Hence, roots of low-P NM and VAM plants were 30% and 65% more efficient at acquiring P, respectively, than roots of high-P NM plants (averaged over 40 to 63 DAP). The beneficial influence of VAM infection on the efficiency of plant P acquistion was absent under high P nutrition, possibly because high abiotic P supply to plants can produce a growth inhibition of external hyphae of *G. fasciculatum* associated with these plants (Abbott et al., 1984). The efficiency of P acquisition by roots increased over the harvest interval, reflecting increased growth of roots or external hyphae and roots, depending upon treatment.

The effects of P nutrition and VAM infection on root respiration at 46 DAP are shown in Figure 3. Although gas fluxes may be expected to decrease within hours of root excision (Bloom and Caldwell, 1988), evolution of CO_2 and ethylene (McArthur and Knowles, 1992) by all root samples in our study was linear over the 5-h incubation interval. While it is likely that root washing and excision perturbed the physiology of these roots, relative differences in respiration in response to treatments, as depicted in Figure 3, are likely representative of those for actual root respiration. Thus, the respiration of roots from low-P plants was 26% lower than that of roots from high-P plants (Fig. 3 and inset). A similar difference between low-P and high-P plants for root respiration was reported by Baas et al. (1989), who measured respiration for *Plantago* polarographically and without root excision. In contrast to the *Plantago* study, which indicated a substantial increase in respiration in response to root infection by *G. fasciculatum*, VAM infection of potato roots did not significantly alter root respiration. This discrepancy likely reflects damage to the external hyphae during washing in our study and, possibly, disruption to intraradical hyphae also. In this regard, metabolic activity for intraradical hyphae and vesicles, as demonstrated by fungal dehydrogenase activity (see below), was still apparent for some time after root excision. Moreover, VAM roots had a 24% higher level of reduced-N than NM roots, which indicates a higher potential for respiration had existed in VAM roots relative to NM roots (Table III).

In spite of the reduced root respiration of low-P plants, the specific activity of vanadate-sensitive ATPases in root microsomes was 13% higher for low-P plants than that for high-P plants (Table V). VAM infection had no effect on the specific activity of

Figure III-3. Time course of CO_2 production by excised roots from 46-d-old potato plants grown with 0.5 mM (○, ●) or 2.5 mM (□, ■) P and inoculated with NM (○, □) or VAM (●, ■) (*G. fasciculatum*) clover inoculum. Roots were incubated in Mes (Tris) buffer (pH 6.5) at 23°C for 5.0 h. F-values for the main effects of $Time_{linear}$, [P], and the interaction $Time_{linear}$ x [P] were significant at the 0.01, 0.01, and 0.01 levels, respectively. Inset: Root respiration rate during incubation.

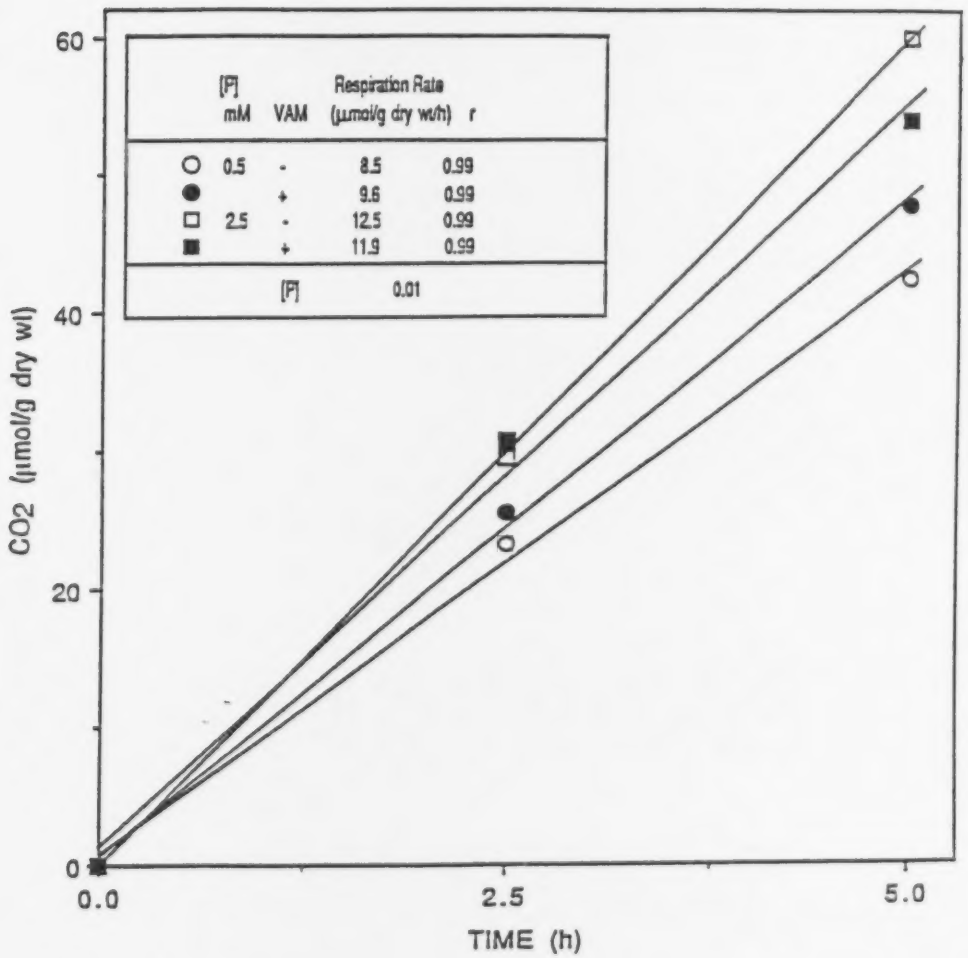

[P] mM
VAM
Respiration Rate (µmol/g dry wt/h)
r
0.5 - 8.5 0.99
+ 9.6 0.99
2.5 - 12.5 0.99
+ 11.9 0.99
[P] 0.01
60
40
20
0
CO2 (µmol/g dry wt)
0.0
2.5
5.0
TIME (h)

Table V. *Effect of a Vesicular-Arbuscular Mycorrhizal (VAM) Fungus and P Supply on the activity of ATPases and Apases of roots of Potato Plants*

Plants were inoculated with nonmycorrhizal or VAM (*Glomus fasciculatum*) clover-pot-culture and fertilized with a complete nutrient solution with either 0.5 mM or 2.5 mM P. Results for plants harvested at 38, 47 and 53 DAP were pooled.

Treatment		Microsomal ATPase		Microsomal Apase[a]				Supernatant Apase[b]	
		+Molybdate		+Molybdate					
[P]	VAM	μmol P $mg^{-1}h^{-1}$	μmol P g^{-1}fresh wt h^{-1}	μmol P $mg^{-1}h^{-1}$	μmol P g^{-1}fresh wt h^{-1}	μmol P $mg^{-1}h^{-1}$	μmol P g^{-1}fresh wt h^{-1}	μmol P $mg^{-1}h^{-1}$	μmol P g^{-1}fresh wt h^{-1}
0.5	-	65.0	9.5	10.6	1.7	24.5	4.0	68.4	81.0
	+	59.9	14.6	9.1	2.2	21.7	5.4	45.6	85.0
2.5	-	58.7	8.6	9.9	1.5	21.2	3.2	65.4	74.6
	+	52.3	12.2	8.2	1.9	19.9	4.6	43.2	74.0
[P][c]		0.05[d]	0.01	0.05	0.05	0.05	0.05	0.01	0.01
VAM		ns	0.01	0.05	0.01	0.05	0.01	0.01	0.01
[P] x VAM		ns	0.10	ns	ns	ns	ns	0.10	0.01

[a], Roots were harvested at 47 and 53 DAP for microsomal Apase. Molybdate decreased activity ($P < 0.01$). [b], Roots were harvested at 47 DAP for supernatant Apase. Molybdate decreased activity (data not shown, $P < 0.01$).

[c], Sources of variation. [d], Significance levels for indicated sources of variation (ns = not significant).

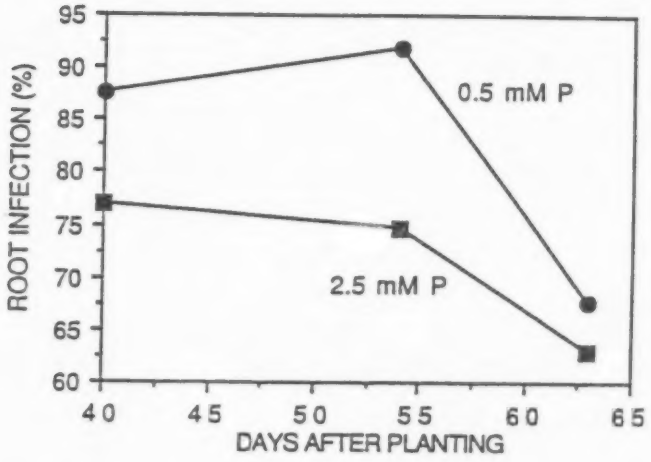
ATPase; however, because VAM roots had significantly more protein, ATPase activity on a fresh weight basis was 48% higher for microsomes of VAM roots than those of NM roots. Root protein content was not affected by P nutrition and, hence, the influence of low-P on ATPase activity on a fresh weight basis was similar to that observed for specific activity. Although molybdate was included in ATPase assays to inhibit non-specific Apases, microsomal Apase activity could account for as much as 17% of the ATP hydrolysis recorded in our study (Table V). In this regard, Apase activity of microsomes and supernatants was inhibited by 57% (Table V) and 86% (data not shown, $P < 0.01$), respectively, by 1 mM molybdate, in contrast to the 14% inhibition by molybdate observed in assays for microsomal ATPase activity (see Methods). Apparently, some supernatant Apases were trapped in microsomes during processing, in spite of repeated washing of the pellet. However, the specific activity of Apases in supernatants was 2.5-fold greater on average than that in microsomal suspensions and 18 times greater on a fresh weight basis (Table V). In general, the contribution of these Apases to ATP hydrolysis under our assay conditions appeared to be minor and likely did not contribute significantly to treatment effects on ATPase activity.

The effects of P nutrition and VAM infection on root Apase activity in the absence of molybdate are also shown in Table V. The specific activity of Apases in supernatants and microsomes of low-P plants was 5% and 12% greater, respectively, than that of high-P plants. Associated with improved P nutrition and greater protein content of VAM plants, the specific activity of Apases in supernatants and microsomes of VAM roots was 51% and 10% lower, respectively, than that of NM roots. Because of the greater protein content in VAM roots, however, microsomal Apase activity of VAM roots on a fresh weight basis was 37% higher than that of NM roots. On the other hand, supernatant Apase activity of VAM roots on a fresh weight basis was only marginally increased in low-P plants, similar to that reported by others (Capaccio and Callow, 1982; Dodd et al., 1987).

VAM Root Morphology

Infection of potato roots by *G. fasciculatum*, as a function of time and level of P supply, is shown in Figure 4. Although the level of infection was extensive throughout the harvest interval, percent infection declined from 54 to 63 DAP. This reduction occurred during rapid tuber growth from 40 to 63 DAP and could be attributed to diversion of plant nutrients away from the fungus (Schwab et al., 1991). The extent of root infection was greater for plants grown with 0.5 mM P than with 2.5 mM P, indicating that P deficient plants were more susceptible to infection. Low-P roots have a

Figure III-4. Time course of root infection by *G. fasciculatum* for potato plants grown with 0.5 mM or 2.5 mM P. F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, [P], and the interaction $Time_{deviations}$ x [P] were significant at the 0.01, 0.01, 0.01 and 0.05 levels, respectively.


95
90
85
80
75
70
65
60
ROOT INFECTION (%)
0.5 mM P
2.5 mM P
40
45
50
55
60
65
DAYS AFTER PLANTING

lower capacity for ethylene generation and also have less extracellular peroxidase activity than high-P roots (McArthur and Knowles, 1992). Reduction in such activities may be indicative of lower capacity for the induction of a defense response by the plant, resulting in higher levels of infection.

Root hair development was greater on NM roots than on VAM roots (Fig 5A and 5B). Incubation of excised VAM roots in MTT solution demonstrated that both extraradical and intraradical VAM structures had considerable dehydrogenase activity (Fig. 5B). Staining was uneven in uninfected root-sections, where MTT was reduced to the insoluble, blue formazan product. This irregular pattern of staining probably reflects the low permeability of intact plant cells to MTT (Altman, 1976). In the infected sections of roots, lipids in spores, hyphae, and vesicles stained red after incubation with MTT, due to the lipid-soluble nature of the formazan product. For the VAM fungus, the majority of formazan product appeared in root vesicles and spores, thus characterizing them as active sites of metabolism. Little formazan product appeared in external hyphae, possibly reflecting damage incurred during root washing.

A high proportion of parenchyma cells in root cortices was colonized (Fig. 5C), with many arbuscules interfacing cells directly adjacent to the endodermis. The fine, dichotomous hyphae of arbuscules in colonized cells often filled the cell lumen (Fig. 5D), facilitating maximum contact with the protoplast and favoring nutrient exchange (Smith and Smith, 1990). The cryostage scanning electron micrograph in Figure 5E shows various intracellular VAM hyphae in close association with a freeze-etched cell protoplast. Such host cells exhibit ultrastructural evidence of heightened metabolic activity (Cox and Tinker, 1976; Holley and Peterson, 1979).

Vesicles predominated over arbuscules and other VAM structures in this study, and over 400 vesicles per cm of root were occasionally observed. Transversely-fractured roots (Fig. 5F) illustrate the physical impact of these lipid-filled vesicles on host cells. Transmission electron micrographs of replicas of freeze-fractured vesicles displayed an ultrastructure similar to that previously described from thin sections (Holley and Peterson, 1979) and provided additional details of the ultrastructure of organelles and vesicular architecture (Fig. 6). Fenestrated cisternae of endoplasmic reticulum were arranged in stacks and were often in close association with one of the many nuclei present. Nuclear pores were prominent, and these displayed an uneven arrangement. Lipid globules were numerous, while few mitochondria were observed (Fig. 6A). Vesicle walls appeared to be trilaminate in replicas (Fig. 6B), and this was later confirmed with ultrathin sections. The ultrathin sections (not shown) also demonstrated a very

Figure III-5. Dark field and scanning electron micrographs of potato roots infected with the VAM fungus, *G. fasciculatum*. A. Dark field micrograph of a NM root, with an external VAM hypha for comparison of root hair and hyphal length, root hair (r), extraradical hypha (E) (Magnification, X30). B. Dark field micrograph of a VAM root and its associated extraradical hyphae (E). Roots were incubated for 20 h in Hepes buffer containing MTT. The open arrow indicates blue formazan crystals in a NM root section. Arrows and the curved arrow indicate red formazan in vesicles and a spore, respectively, (Magnification, X30). C. Scanning EM of a transverse fracture of a root illustrating arbuscules (A) in the cortex and exclusion of the VAM fungus from the stele by the endodermis (e); intracellular hyphae (H), (Bar = 40 μm). D. Arbuscular (A) and intracellular (H) hyphae within a cortical cell (Bar = 10 μm). E. Cryostage scanning EM of a root showing intracellular (H) and arbuscular hyphae (A) admidst cytoplasmic contents (c) of the root cell (Bar = 10 μm). F. Scanning EM of a transverse fracture exposing numerous vesicles and showing exclusion of the fungus from the stele (Bar = 40 μm).

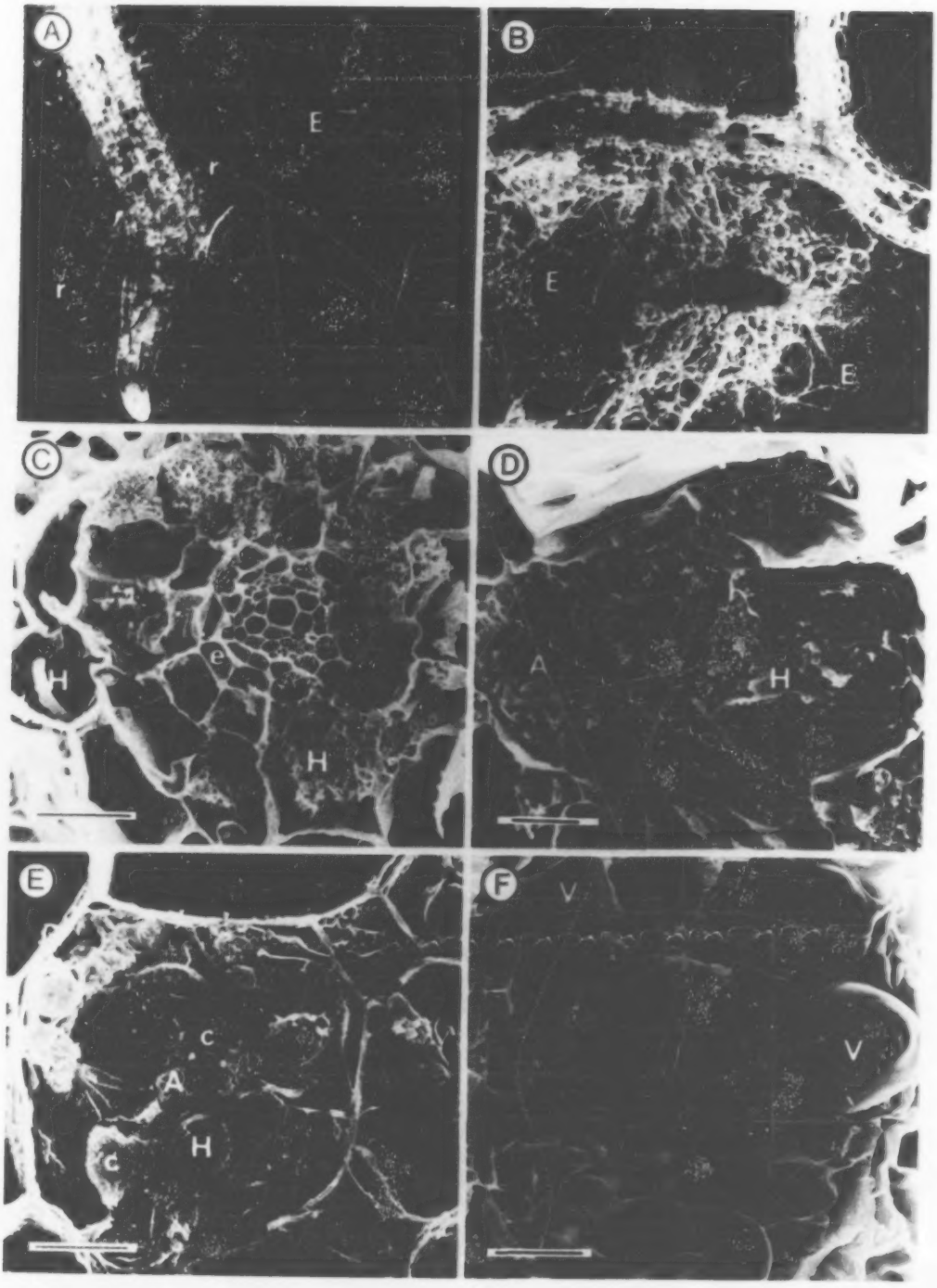
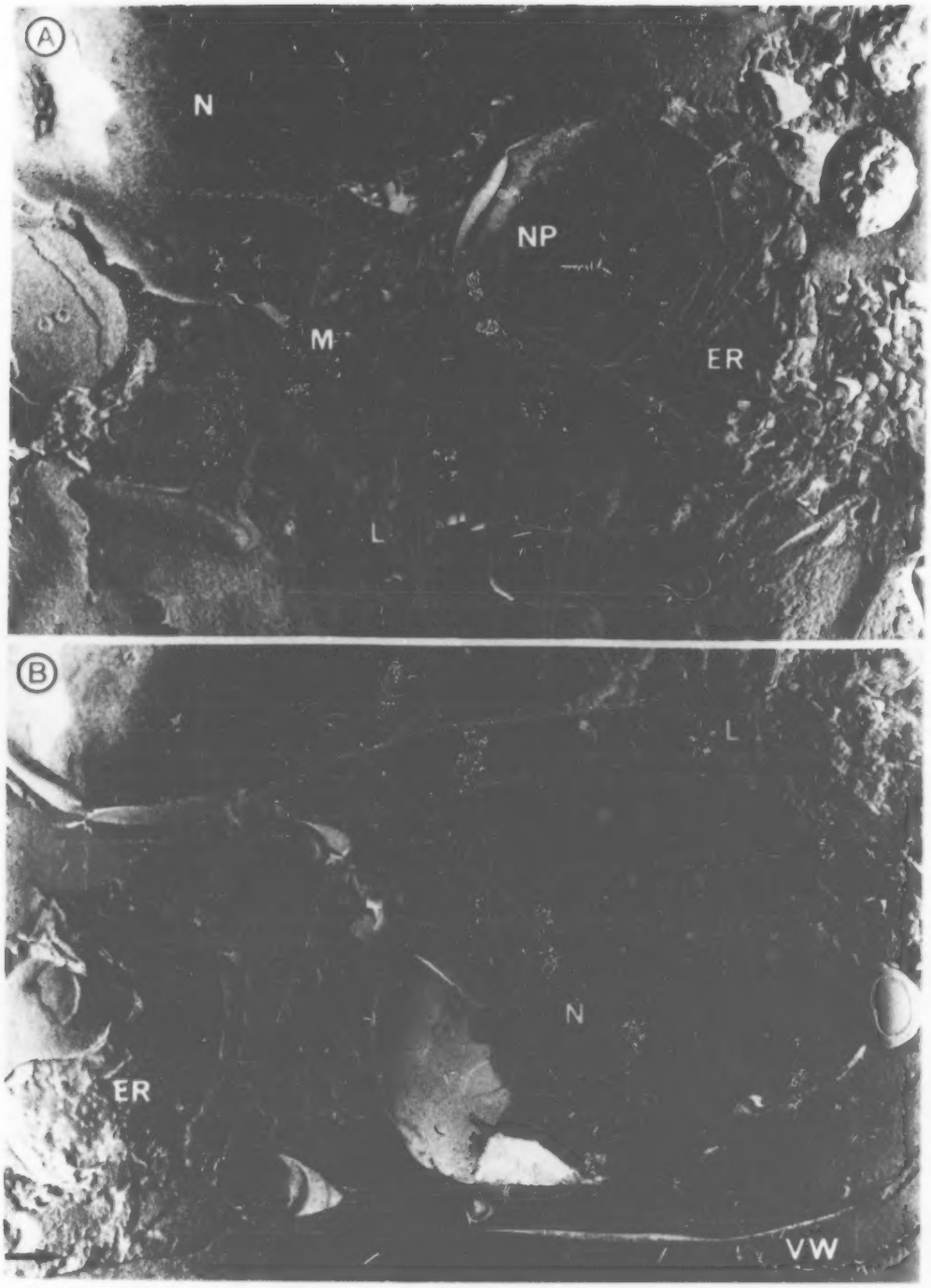

A
E
r
r
B
E
E
C
e
H
H
D
A
H
E
c
A
H
c
F
V
V

Figure III-6. Transmission electron micrographs of replicas from freeze-fractured vesicles of potato roots infected with the VAM fungus, *G. fasciculatum*. A. endoplasmic reticulum (ER), lipid droplet (L), mitochondria (M), nucleus (N), nuclear pores (NP), (Magnification, X26,000). B. endoplasmic reticulum (ER), lipid droplet (L), nucleus (N), vesicle wall (VW), arrow indicates direction of carbon-shadowing, (Magnification, X26,000).


A
N
NP
M
ER
L
B
L
N
ER
VW

large number of lipid globules of various sizes in each vesicle, some of which were associated with mitochondria (cristae evident).

DISCUSSION

Our results characterize a pronounced P deficiency in NM and VAM potato plants grown with 0.5 mM P. Restricted leaf canopy development (Table I) and dry matter accumulation (Fig. 1) due to low abiotic P supply were prominent indicators of plant stress. Moreover, the accumulation of relatively high levels of non reducing sugars and nitrates in shoots and roots indicated that these plants were neither carbohydrate- nor N-starved (Tables II and III). On the other hand, the P status of low-P plants was clearly inferior to that of high-P plants (Fig. 2 and Table IV). These responses of potato plants to low abiotic P supply were similar to those generally attributed to P deficiency stress caused by inadequate P supply (Fredeen et al., 1989; Harris, 1979) or short-term P deprivation (Cogliatti and Clarkson, 1983; Goldstein et al., 1988; Rufty et al., 1989).

A general restriction of plant metabolism by P deficiency stress was apparent by a number of metabolic indicators. Leaf nitrate reductase activity and root respiration were substantially lower for low-P plants than for high-P plants (Fig. 3). Since respiration is often proportional to cell growth, and nitrate reduction constitutes a significant investment of energy for plant growth (Johnson, 1990), the results characterize a decrease in the amount of respiratory energy devoted directly to growth processes as a result of P deficiency stress. Indeed, the lower dry matter accumulation for low-P NM plants between 40 and 63 DAP (Fig. 1) implies minimal investment of energy from respiration into processes which directly contribute to growth. Hence, most of the energy derived from root respiration of low-P plants was likely expended to maintain existing biomass. Energy-dependent maintenance processes such as the transport of nutrients across cell membranes and against electrochemical gradients, including the re-assimilation of leaked ions to balance ion efflux (Leonard, 1985; Baas et al., 1989; Johnson, 1990) may be a relatively greater sink for metabolic energy under P stress. The greater efficiency of low-P plants in acquiring what little P was available (Table IV) suggests that meeting the energy requirement for P uptake was a more significant energy-dependent component of the root's total respiratory activity in low-P plants. Our results on the efficiency of P acquisition are consistent with observations of an enhanced root P-uptake system in potato under conditions of P deficiency (Cogliatti and Clarkson, 1983).

In spite of the inhibitory effects of P deficiency stress on overall growth and metabolism, specific physiological processes were actually stimulated by low abiotic P supply. For example, the allocation of photoassimilates (Table I) and P to roots (Table IV) appeared to be less restricted by low abiotic P supply than that for shoots or tubers. This indicates an altered pattern of dry matter partitioning favoring root growth and, thus, mineral acquisition (Cogliatti and Clarkson, 1983). Vanadate-sensitive ATPase activity in root microsomes was stimulated in response to P deficiency stress (Table V). This response may also be relevent to enhanced P acquisition, since root plasma membrane-ATPases (which are also vanadate-sensitive) are considered responsible for acid secretion, which may increase P availability in the rhizosphere (Clarkson, 1985). These ATPases also provide the proton motive force required by carrier proteins to facilitate active uptake of nutrients such as $H_2PO_4^-$ across membranes (Leonard, 1985). Soluble Apase activity (intracellular plus extracellular) in roots was also enhanced in response to P deficiency stress (Table V). In terms of improving the plant's P nutrition, a role for soluble excreted Apases in scavenging organic-P from the rhizosphere has been suggested (Goldstein et al., 1988; Goldstein et al. 1989). Moreover, an increase in the intracellular Apases may allow for more efficient utilization of P in primary metabolism (Duff et al., 1989). While further investigation is required to establish a stronger connection between the low-P-induced increase in the efficiency of P acquisition and the activities of microsomal ATPases and soluble Apases, our results indicate that such an approach is warranted.

Collectively, the changes in plant metabolism in response to low abiotic P supply that we have characterized may constitute part of a "phosphate starvation rescue system" in higher plants (Goldstein et al., 1988; Lefebvre et al., 1990). Such changes in root physiology during the development of P deficiency are generally considered to be adaptive and to improve plant acquisition or conservation of P (Cogliatti and Clarkson, 1983; Clarkson, 1985; Goldstein et al., 1989; Lefebvre et al., 1990). The actual contribution of these physiological changes to the greater efficiency of P acquisition characterized for low-P plants in our study, however, is difficult to estimate. On the other hand, the contribution of the VAM symbiosis to plant P nutrition was more easily quantified.

The growth response of low-P plants to VAM infection (Fig. 1) and the P status of these plants (Table IV) demonstrated that the mycorrhizal symbiosis enhanced plant P nutrition beyond that which could be derived solely through adaptation of the plant grown independently. However, the VAM symbiosis was capable of only partially alleviating P deficiency stress and could not compensate for low abiotic P supply. In a separate study,

it was demonstrated that the VAM symbiosis could completely compensate for a low abiotic P (1.5 mM) supply which was one-half that of the high P supply (3.0 mM) (unpublished results). In the current study, the extent to which VAM infection improved plant growth was clearly a function of its P contribution (Table IV and Fig. 2). Moreover, the VAM benefit was limited by the amount of P available in low-P pots. A conservative estimate of 81% for the P acquisition efficiency for low-P VAM plants (root P was not included) indicates that mycorrhizal roots were able to account for most of the available P in the soil. Although more efficient P scavenging would likely be attributable to external hyphae (Fig. 5A and 5B), increased P absorption due to VAM-stimulated root growth may also be important (Fig. 1C).

Coincident with the effects on plant P nutrition, the fungal symbiont appeared to have some influence on plant allocation of dry matter and leaf development. For example, leaf expansion was stimulated relatively more than biomass accumulation (Fig. 1) and hence, a main effect of VAM on LAR was evident (Table I). Enhanced leaf expansion for VAM plants was likely related to the fact that both P allocation to shoots (Table IV) and nitrate reductase activity in leaves (Table III) were increased in VAM plants, even in the absence of a growth response to the VAM symbiosis for high-P plants. Possibly reflecting an enhanced sink strength in roots as a result of symbiotic activity (Snellgrove et al., 1982; Baas et al., 1989), the relative increase in root dry weight gain in response to the symbiosis was more than that for shoots. This was demonstrated by a lower S_W/R_W for VAM plants than for NM plants at 63 DAP, and in spite of a higher shoot mass for low-P VAM plants than for low-P NM plants.

Generally, the negative influence of VAM infection on the specific activity of root ATPases and Apases was consistent with an improved P nutrition in plants via the VAM symbiosis (Table V). The promotion of these enzyme activities on a fresh weight basis was likely a direct consequence of the enhanced plasma membrane surface area of cells due to arbuscular growth (Fig. 5C and 5D) (Cox and Tinker, 1976). Enzymatic studies using cytochemical techniques to characterize the presence of ATPases and Apases also showed enhanced activities of these enzymes, specifically at interfaces between intraradical VAM structures and the plasma membranes of colonized root cells (Gianinazzi-Pearson et al., 1991). While the contribution of fungal ATPases and Apases to these activities in VAM roots could not be estimated in our study, we did find some Apase activity in soluble extracts of isolated chlamydospores and vesicles (data not shown). On the other hand, during a preliminary characterization of Apases from NM and VAM roots by native PAGE, a soluble Apase activity which could be attributed to the VAM fungus was not detected in extracts from VAM roots.

In this and a related study (McArthur and Knowles, 1992), potato plants grown with low abiotic P supply were more susceptible to infection by *G. fasciculatum* than those grown with a high abiotic P supply. Also, the capacity for ethylene production and extracellular peroxidase activity in roots increased proportionally with increasing P supply, while VAM infection of roots substantially inhibited ethylene production. We further demonstrated that the VAM inhibition of 1-amino cyclopropane-1-carboxylic acid (ACC) oxidase activity could be attributed to a soluble, possibly phenolic, substance in VAM root leachates. Hence, these results indicated a possible link between plant mineral physiology and compatibility of plants to VAM fungi. In the present study, P deficiency stress influenced metabolism in a fashion which might have limited the resources available for induction of a defense response to VAM infection and, thus, may have been a contributing factor to enhanced root susceptibility. However, inhibition of ACC oxidase in VAM roots was also present for plants grown with high abiotic P supply and exhibiting a high rate of root respiration (Fig. 3). Further investigations into a link between plant compatibility to VAM fungi and P starvation-induced metabolism might consider the possible influence of P nutrition on root susceptibility to VAM infection through changes to root phenolic metabolism (Siqueira et al., 1991).

In summary, the growth and yield of potato were shown to be very sensitive to inadequate P nutrition in this study. The partitioning of dry matter to tubers was most affected by P nutrition in our study (Fig. 1), likely as P deficiency stress developed and increased during the period when the requirement for P was greatest, i.e. during tuberization and bulking (Nelson et al., 1947; MacKay et al., 1988). Our results underscore the importance of preventing P deficiency during growth of potato and suggest a potential benefit from the VAM symbiosis under appropriate field conditions. It should be noted that, even under a P regime in which no growth benefit was obtained, no detrimental effect of the VAM infection was observed. Finally, we suggest that a P starvation-induced increase in susceptibility of plants to VAM fungi be interpreted as another component of P starvation-induced metabolism. Elucidation of the metabolic basis for enhanced susceptibility to VAM infection may provide an improved understanding of developmental and physiological patterns observed in plants in response to various sources of stress.

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Chapter IV

Changes to Plant Growth, Mineral Physiology and Root Acid Phosphatases of Potato in Response to Differential Phosphorus Nutrition and a Vesicular-Arbuscular Mycorrhizal Fungus[3]

Introduction

As a major nutrient ion, phosphate is intimately involved in plant growth and metabolism, and there are numerous examples by which P nutrition influences physiological processes of plants (Bieleski and Ferguson, 1983; Clarkson, 1985). In roots for example, P deficiency induces high ^{2}Apase activity and an enhanced phosphate uptake and transport capacity (Bieleski and Ferguson, 1983; Cogliatti and Clarkson, 1983; Goldstein et al., 1988; Duff et al., 1991). Such alterations to root physiology appear to play an integral part in improving the plant's efficiency of P uptake and utilization (Duff et al., 1991; Clarkson, 1985; Goldstein et al., 1989; Lee, 1988). For the majority of terrestrial plants, P deficency also enhances the compatibility of plants towards VAM fungi (Koide and Schreiner, 1992; McArthur and Knowles, 1992) and, through establishment of such a symbiosis, plants may further increase the efficiency of P acquisition by roots (Harley and Smith, 1983; McArthur and Knowles, 1993). Since the concentration of available P in solution-phase of soil is naturally very low and as P is relatively immobile in soils, contributions of P by VAM fungi to plant P nutrition may be both ecologically and agronomically significant (Black and Tinker, 1977; Abbott and Robson, 1982; McArthur and Knowles, 1991).

It is widely recognized, however, that the extent of benefit to plant growth and the physiological response of plants to VAM fungi depend upon the P-status of the host plant (Abbott and Robson, 1982; Smith and Gianinazzi-Pearson, 1988). In this regard, some degree of P-deficiency stress in the host appears essential for the growth of the VAM fungi and maintenance of the symbiosis (Koide, 1991) and, thus, limits the agronomic importance of the VAM symbiosis to crops that are, at present, highly fertilized with P. Although the mechanism by which increasing abiotic P supply inhibits the symbiosis is not understood, there are numerous processes in plant metabolism through which P nutrition could exert an influence on host compatibility (Koide and Schreiner, 1992; McArthur and Knowles, 1992). In this regard, nutrient exchange between symbionts is

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[3] A version of this chapter has been prepared for submission. McArthur & Knowles 1992 Physiologia Plantarum

considered to be strongly influenced by P nutrition and to play a central role in host - symbiont compatibility (Smith and Gianinazzi-Pearson, 1988; Schwab et al., 1991).

Recently, Schwab et al (1991) proposed a model for symbiotic nutrient exchange in which Apase activity, induced by P-deficiency stress, would function in the interfacial (extracellular) matrix to promote the operation of a triose-P translocator, thus facilitating the exchange of nutrients between symbiont and host. Further implicating Apases in the process of nutrient exchange, cytochemical studies of VAM roots indicate not only increased ATPase activity, but also a high level of Apase activity in the interfacial matrix between arbuscular hyphae and root cells (Gianinazzi-Pearson et al., 1991). The low pH produced at this interface by active plasma membrane ATPases would promote both Apase activity and P uptake. Although an improved P-status of plants as a consequence of the VAM symbiosis might be expected to diminish root Apase activity, Dodd et al (1987) found that extracellular Apase activity of wheat and onion roots was stimulated in response to infection by various VAM species, thus indicating that this may be a general phenomenon for the VAM symbiosis. Furthermore, electrophoretic analysis of root Apases for several cultivars of bean has demonstrated enhanced activity for one isozyme in response to root infection by *G. etunicatum* (Pacovsky et al., 1991). Hence, the presence of some isozymes might be reduced by a VAM improvement of P nutrition, but the synthesis of others, required for nutrient exchange, might be induced. Although evidence demonstrating a role for Apases in P uptake by NM plant cells is equivocal (Lee, 1988; Lefebvre et al., 1990), in the mycorrhizal system, Apase activity in the interfacial matrix of VAM roots could be a significant factor in P uptake by cells.

Previously, we had characterized the physiological response of potato roots to P nutrition and the VAM symbiosis in terms of various components of the plant defense response, and had suggested that the plant's susceptibility to VAM infection might somehow be linked to the physiological response of plants to P-deficiency stress (McArthur and Knowles, 1992). The present report decribes additional results from that study for the effects of differential P nutrition and *G. fasciculatum* on the growth and mineral physiology of potato. Of particular interest were changes in the root protein profile and Apase (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.2) activity of plants, which were induced by the symbiont and P-deficiency stress. Such information is useful in evaluating a potential role for Apases in symbiotic nutrient-transfer. The results herein further characterize the physiological response of potato to the VAM symbiosis, but do not support the hypothesis for a role of a VAM-induced Apase

isozyme or enhanced Apase activity in the exchange of nutrients between symbiont and host.

MATERIALS AND METHODS

Inocula

Inocula for NM and VAM (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) treatments consisted of soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved sand:soil (v/v 3:1) medium for 90 d. Plants were grown in 2-L pots and were fertilized weekly with 50 mL of a modified (minus P) Hoagland solution. Roots of NM clover plants were uninfected, whereas those of VAM plants were 80% infected and had many vesicles. Few mature chlamydospores were present on either the surface of roots or in the soil of the VAM inoculum. To quantify infection, roots were cut into 1-cm lengths and were stored in FAA solution (5% formalin, 5% acetic acid, 45% ethanol) until further processing by clearing and staining (Phillips and Hayman, 1970). Percent infection was assessed using the gridline intersect method (Giovanetti and Mosse, 1980) with 100 counts per slide, and five (clover) or three (potato [*Solanum tuberosum* L. cv Russet Burbank]) slides per plant.

Plant Growth Conditions

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface-sterilized with 1.0% (w/v) sodium hypochlorite (3 min) and rinsed thoroughly with distilled H_2O. Single-eye seedpieces (5.0 g) were cut from the mid-region of the tubers and rinsed. After air-drying for 1 h, the seedpieces were planted in vermiculite and sprouted in the dark for 10 d at 27°C (95% RH).

Single-sprout seedpieces were blocked for sprout length prior to transplanting onto NM or VAM soil inoculum (50 g fresh weight pot^{-1}) in 20-cm diameter pots (1 seedpiece pot^{-1}). Each pot contained 3.4 kg (air-dry) of an autoclaved sand:soil (6:1 v/v) medium (5 µg/g $NaHCO_3$-extractable P). Pots were placed in a growth chamber set at 25/20°C (day/night) with a 16-h photoperiod. A PPFD of 540 µmol $m^{-2}s^{-1}$ was provided by fluorescent and incandescent lights maintained at 14 cm from the shoot tips. Each pot received nutrients as indicated below. In the first week, each pot received 50 mL of nutrient solution three times (40 mM KNO_3; 20 mM $Ca(NO_3)_2$; 20 mM $MgSO_4$; 185 µM H_3BO_3; 36.5 µM $MnCl_3$; 0.3 µM $ZnSO_4$; 1.3 µM $CuSO_4$; 0.065 µM H_2MoO_4; 2 mg/L Fe-EDDHA [pH 6.0]), then 100 mL with added P (0.5, 2.5, or 5.0 mM NaH_2PO_4) was given three times per week thereafter.

Experimental Design and Analytical Methods

Treatments consisted of four harvest dates (21, 28, 35, and 42 DAP), three levels of nutrient solution P (0.5, 2.5, and 5.0 mM), and two inocula (NM or VAM) arranged factorially in a randomized complete block design (three blocks).

Roots of intact plants were gently washed in running water, rinsed in cold deionized, distilled H_2O, and stored briefly (max 30 min) in cold deionized, distilled H_2O (4°C). The roots were then blotted dry with filter paper, and groups of fine roots from the midregion of each root system were cut into 1-cm lengths. Excised roots were placed in duplicate 15-mL disposable test tubes (0.5 g fresh weight $tube^{-1}$) containing 5 mL of 50 mM Mes (Tris) buffer (pH 6.5) with 25 mM NaCl, and incubated for 2.5 h at 25°C. Leachates were filtered through Miracloth (Calbiochem) and collected for assay of extracellular Apase activity. The remaining roots for each plant were frozen (-20°C) for later extraction. Total root Apase activity was extracted (mortar and pestle) at 4°C in Mes (Tris) buffer (50 mM; pH 6.5) containing 2.5 mM $MgSO_4$ (1 g fresh weight roots to 10 mL buffer). Extracts were filtered through a double layer of miracloth and centrifuged at 15,000*g* for 20 min (4°C). Pellets were discarded and supernatants were used for determinations of total Apase activity.

Apase activity was determined at A_{410} with a nitrophenol standard (Gianinazzi-Pearson and Gianinazzi, 1976). A 250-µL aliquot of root filtrate (for root extracts, 50 µL) was added to 1 mL of 50 mM sodium acetate buffer (pH 5.5) containing 2.5 mM *p*-nitrophenyl-phosphate (Sigma), 2.5 mM $MgSO_4$ and 1 mM $CaCl_2$ and incubated at 25°C The reaction was stopped after 15 min (for extracts, 5 min) with 1 mL of 1 N NaOH. Protein was assayed by the method of Bensadoun and Weinstein (1976) with BSA as a standard. Apase activity was expressed on a gram fresh weight or a milligram protein basis. Plant tissues were divided into shoots, tubers and roots and lyophilized for dry weight and mineral analyses. From these variables, LAR (LA/W), RGR (1/W x dW/dt), RSAR ($1/R_W$ x dP/dt x 1/P-available, P-available = P supplied between harvest dates, including $NaHCO_3$-extractable soil-P and initial plant-P), R_W/W, SAR ($1/R_W$ x dP/dt), S_W/W, T_W/W, and ULR (1/LA x dW/dt) (Hunt, 1982).

Frozen (-20°C) root filtrates and extract supernatants were thawed and centrifuged at 100,000*g* (4°C) for 1 h. Protein was concentrated via ultrafiltration with a Centricon-10 (Amicon Canada Ltd, Oakville, Can.). Native PAGE was performed on a PhastSystem (Pharmacia) with an 8-25% PhastGel gradient and separation technique 120. Approximately 0.1 µg of protein per treatment was loaded onto a PhastGel set with native buffer strips. Gels were run for 200 Vh at 15°C, and then stained for Apase activity for 20 min at 37°C in the dark by the method of Wendel and Weeden (1989).

SDS-PAGE was performed using a Bio-Rad mini-gel apparatus (20°C) and the method of Laemmli (1970). The final acrylamide monomer concentration in 0.75 mm thick slab gels was 11% (w/v) for the separating gel and 4% (w/v) for the stacking gel. All samples (25 µg soluble protein for root extracts and 2 µg for leachates) and molecular weight markers were boiled (100°C) in 1% SDS, 20% *B*-mercaptoethanol, 30% glycerol, and 1% bromophenol blue for 3 min prior to loading. Gels were run at 150 V constant voltage until the tracking dye reached the end of the gel. Protein bands were stained with Coomassie blue or silver ('oxidized' protocol of Wells and Cordingley, 1991). For the determination of molecular mass using SDS-PAGE, a plot of R_f *versus* log molecular mass was constructed using the following protein standards: myosin (205 kD), *B*-galactosidase (116 kD), phosphorylase B (97 4kD), BSA (66 kD), ovalbumin (45 kD), and carbonic anhydrase (29 kD).

Lyophilized shoots and roots were ground through a Wiley mill (40 mesh) and 100 mg was ashed at 550°C for 12 h. Ash was dissolved in 0.5 mL HCl and made up to 5 mL using 1 N H_2SO_4. Tissue P was determined by the method of Serrano et al (1976). Shoot and root Ca, Mg, and Cu concentrations were determined with a Perkin-Elmer Model 4000 atomic absorption spectrophotometer with $A_{422.7}$, $A_{202.6}$, and $A_{324.8}$, respectively. Flame emission spectroscopy with an $A_{766.5}$ was used to determine shoot and root K levels. To decrease interferences in Ca and Mg determinations, samples were diluted to produce 0.1% La and 0.1% KCl (for Mg) in 1.2 N HCl (Anon, 1979). Leaf area was measured with a LI-COR LI-3100 area meter.

Statistical Analyses

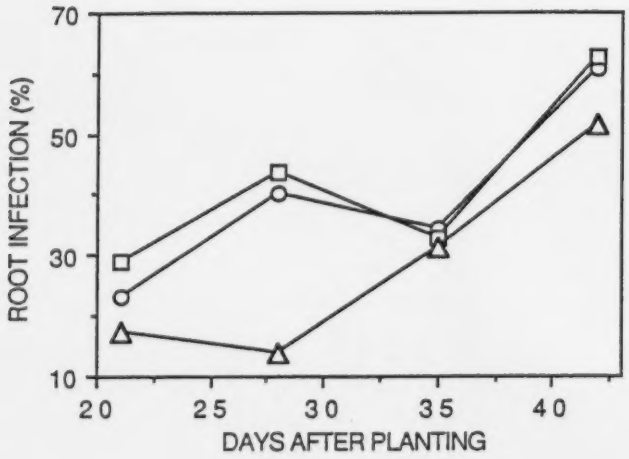
Growth and physiological data were subjected to analysis of variance and, where appropriate, sums of squares were partitioned into individual degree of freedom components of both main effects and interactions. Percent-infection data were arc-sin transformed to achieve homogeneity of variance prior to ANOVA. Based on the ANOVA results, regression analysis was used to derive polynomial models describing the various relationships.

RESULTS AND DISCUSSION

Development of Root Infection

The extent of root colonization by *G. fasciculatum* increased somewhat slowly with time (Fig. 1) and an inhibitory influence of high abiotic P supply (5.0 mM) on intraradical fungal growth was initially evident ($P_{linear} \times Time_{deviation}$, $P < 0.10$, with

Figure IV-1. Time course of root infection by *G. fasciculatum* for potato plants grown with 0.5 (○), 2.5 (□), or 5.0 (△) mM P. F-values for the main effects of $Time_{linear}$, $Time_{deviations}$, $[P]_{linear}$, and the interaction $Time_{deviations}$ x $[P]_{linear}$ were significant at the 0.01, 0.05, 0.01, and 0.10 levels, respectively.


70
50
30
10
ROOT INFECTION (%)
20
25
30
35
40
DAYS AFTER PLANTING

an $LSD_{0.05}$ = 17%). Although not well understood, such inhibition has been attributed to a negative influence of high tissue-P concentrations on physiological factors pertaining to host compatibility (Koide and Schreiner, 1992; McArthur and Knowles, 1992). By 35 DAP, however, the level of infection for high-P plants approached those of low-P (0.5 mM) and mid-P (2.5 mM) plants such that differences at that time and thereafter were absent or marginal. As plant growth with a fixed level of abiotic P supply, even high-P, can eventually cause dilution of tissue-P concentration (see below), it may be that more rapid growth of the symbiont in roots of high-P VAM plants late in this study resulted from a decrease in tissue P-level and hence, increased host compatibility. Morphological detail of this infection process in potato roots has been presented elsewhere (McArthur and Knowles, 1992 and 1993).

Plant Growth and Mineral Physiology

One of the most immediate, observable plant responses to inadequate P-nutrition was a restriction in the development of photosynthetic surface area (Fig. 2). At 21 DAP, low-P NM plants already had 57% less total leaf area than high-P NM plants ($LSD_{0.10}$ = 1.5 dm^2). By 42 DAP, low-P and mid-P NM plants had 60 and 25% lower total leaf area, respectively, than high-P NM plants. Inoculation of roots with *G. fasciculatum* resulted in a promotion of total leaf area, as indicated by a highly significant VAM x $Time_{linear}$ interaction ($P < 0.01$). With the low-P or mid-P treatments, VAM plants had 57 or 25% more total leaf area, respectively, than NM plants. This stimulatory effect of the VAM symbiosis on leaf area development was lost when plants were grown with high P, and the interaction of VAM x [P] was significant ($P < 0.05$). Such P-dependent plant responses to the VAM symbiosis have been typically observed when plants were grown with an abiotic P supply ranging from deficient to adequate, and reflect the extent to which P was limiting to growth (Harley and Smith, 1983; Koide, 1991; McArthur and Knowles, 1993). The physiological influence of P, whether exogenously or biotically supplied, on shoot canopy development is likely exerted through a promotion of plant hydraulic conductivity, growth of lateral apices, and leaf expansion (Fredeen and Terry, 1988; McArthur and Knowles, 1992; Radin and Eidenbock, 1984).

Shoot dry mass accumulation increased linearly between 21 and 42 DAP (Fig. 3), and was very responsive to P nutrition (shoot dry weight = 6.4 + 1.2[P]; r = 0.99). As a result, the shoot dry weight at 42 DAP for low-P and mid-P NM plants was 51% and 25% lower, respectively, than that of high-P NM plants. Inoculation of roots with *G. fasciculatum* promoted shoot growth for plants grown with low-P, as demonstrated by a

42% greater shoot dry weight at 42 DAP for VAM plants than for NM plants. However, increasing abiotic P supply altered VAM fungal interactions with the plant so that no difference between VAM and NM plants was apparent for plants grown with mid-P or high-P.

Root growth was also stimulated by increasing abiotic P supply, although it was one-tenth as responsive to P nutrition as shoot growth (root dry weight = 1.1 + 0.1[P]; r = 0.99) (Fig. 3 insets). Even so, root dry weights for low-P and mid-P NM plants at 42 DAP were still 33% and 24% lower, respectively, than that for high-P NM plants. Unlike shoot growth, *G. fasciculatum* promoted root growth at all levels of P nutrition such that, by 42 DAP, VAM plants had a 22% greater root dry mass than NM plants. Enhanced growth of roots in response to the VAM infection, in spite of the high-P treatment, may reflect the fact that *G. fasciculatum* established a substantial root infection in high-P plants late in the harvest interval (Fig. 1). Such an infection may have increased the 'sink' activity of roots for photoassimilates by creating high, localized-P concentrations (see below) and also by stimulating root metabolic activity (Baas et al., 1989; Cox and Tinker, 1976; McArthur and Knowles, 1993).

In this study, tuber initiation occurred sometime before 28 DAP for NM plants, but after this date for VAM plants, although no significant difference between NM plants and VAM plants in tuber dry mass accumulation was evident (data not shown). Tuber initiation and growth were also dependent upon P nutrition (tuber dry weight = 0.1 + 0.4[P]; r = 0.95) and by 42 DAP, high-P plants had 22- and 1.5-fold greater tuber dry weights, respectively, than that of low-P and mid-P plants. By 42 DAP, high abiotic P supply to plants had a substantial, positive influence on the relative allocation of plant dry matter to tuber growth (Table I). This preferential dry matter partitioning was, in turn, offset by relatively less dry matter allocation to shoots and roots. Analysis of dry matter allocation at 42 DAP showed no significant influence of the VAM symbiosis, similar to our previous results (McArthur and Knowles, 1993).

Plant growth analysis indicated a decrease in RGR of plants with time and a linear increase in RGR in response to increasing abiotic P supply (Fig. 4A and inset). Although total plant dry weight was significantly higher for VAM plants than for NM plants ($P < 0.01$; data not shown), no significant influence of the VAM symbiosis on RGR was evident. In fact, RGR for NM plants was marginally higher than for VAM plants at 28 DAP ($LSD_{0.10}$ = 0.023). Examination of the two main components of RGR, i.e. ULR (Fig. 4B) and LAR (Fig. 4C), indicated that the differences at 28 DAP were primarily due to higher ULR for NM plants than for VAM plants ($LSD_{0.10}$ = 0.34 mg $cm^{-2}d^{-1}$), as LAR was not affected by the VAM symbiosis. A lower ULR for VAM plants than for NM plants

Figure IV-2. Development of total leaf area over time for NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. F-values for the main effects of VAM, $Time_{linear}$, $[P]_{linear}$, $[P]_{deviation}$, and the interactions of VAM x $Time_{linear}$, and VAM x $[P]_{linear}$ were significant at the 0.01, 0.01, 0.01, 0.01, 0.01, and 0.05 respectively.

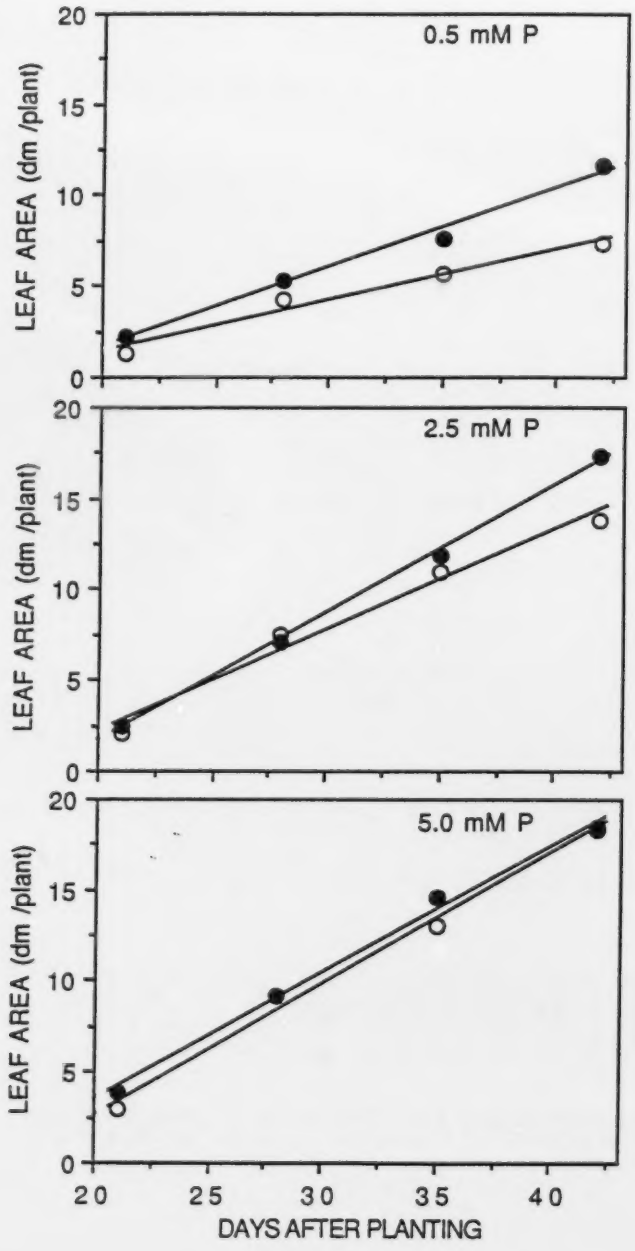

0.5 mM P
2.5 mM P
5.0 mM P
LEAF AREA (dm /plant)
LEAF AREA (dm /plant)
LEAF AREA (dm /plant)
0
5
10
15
20
20
25
30
35
40
DAYS AFTER PLANTING

Figure IV-3. Accumulation of shoot dry mass over time for NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. F-values for the main effects of VAM, $Time_{linear}$, $[P]_{linear}$, and the interactions of VAM x $Time_{linear}$, and VAM x $[P]_{linear}$ were significant at the 0.01, 0.01, 0.01, 0.10, and 0.10 levels, respectively, ($LSD_{0.05}$ = 2.2 g). Insets: Accumulation of root dry mass over time for NM and VAM potato plants grown with 0.5, 2.5, or 5.0 mM P. F-values for the main effects of VAM, $Time_{linear}$, $[P]_{linear}$, and the interaction of VAM x $Time_{linear}$ were significant at the 0.01, 0.01, 0.01, and 0.01 levels, respectively.

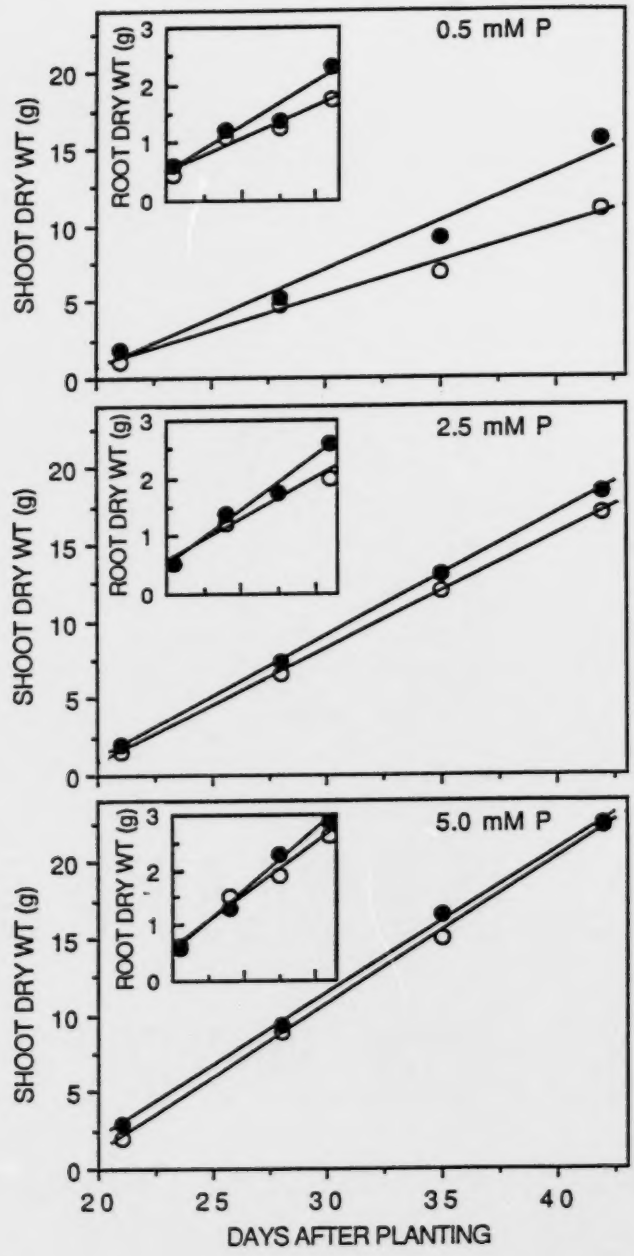

0.5 mM P
2.5 mM P
5.0 mM P
SHOOT DRY WT (g)
ROOT DRY WT (g)
0
5
10
15
20
1
2
3
20
25
30
35
40
DAYS AFTER PLANTING

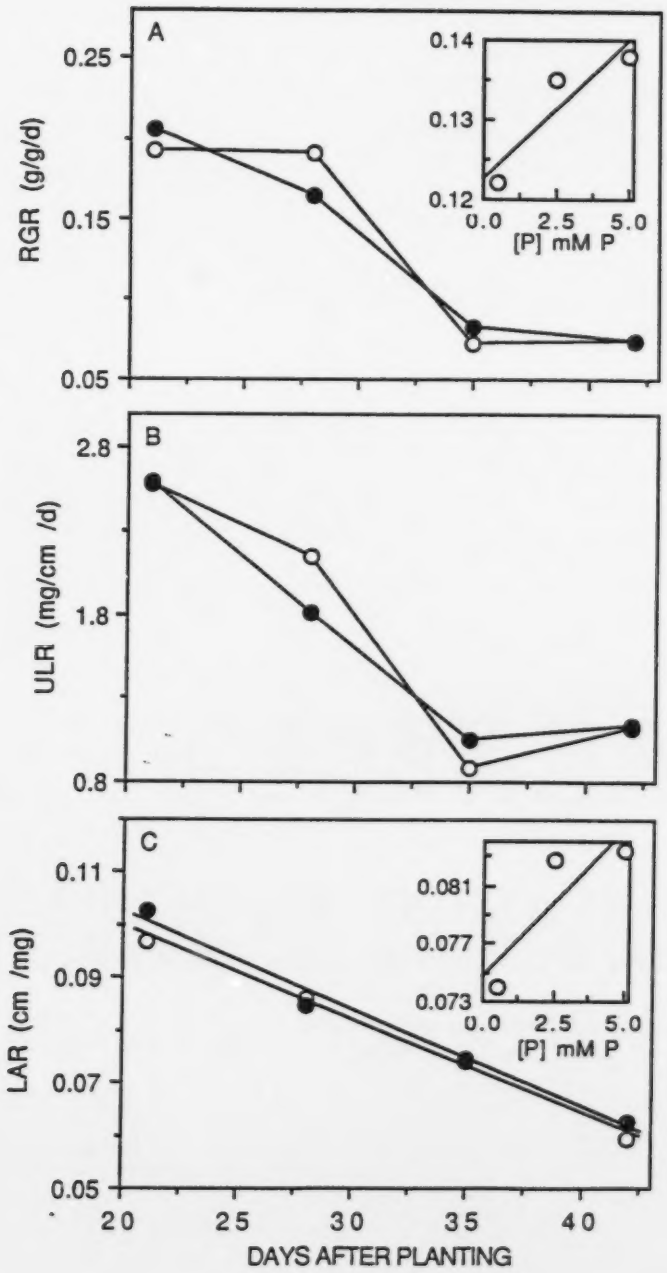
Table I. *Relative Dry Matter Allocation of Nonmycorrhizal and Vesicular-arbuscular Mycorrhizal Potato Plants Grown with Various Levels of Abiotic P-Supply*

Plants were inoculated with control or *Glomus fasciculatum* clover pot cultures, and fertilized with a complete nutrient solution containing 0.5, 2.5, or 5.0 mM P. The data presented is for 42 days after planting.

Treatment		S_W/W^1	R_W/W^2	T_W/W^3
[P] mM	VAM[4]	%	%	%
0.5	-	86.1	13.9	0.0
	+	84.4	12.7	2.9
2.5	-	75.6	9.0	15.5
	+	71.2	10.1	18.7
5.0	-	71.1	8.3	20.6
	+	70.5	9.2	20.3
VAM[a]		ns	ns	ns
P		L	L	L
VAM X P_L		ns	ns	ns
VAM X P_D		ns	ns	ns

[1]shoot:plant dry wt ratio; [2]root:plant dry wt ratio; [3]tuber:plant dry wt ratio; [4]vesicular-arbuscular mycorrhizal.
[a]Sources of variation (L = linear trend). [b]Significance levels or significant trend (L = linear with $P < 0.05$) for indicated sources of variation; ns = not significant.

Figure IV-4. Main effect of time on plant growth indices for NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. A, RGR. F-values for the main effects of $Time_{deviation}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{deviation}$ were significant at the 0.01, 0.10, and 0.05 levels, respectively, ($LSD_{0.10}$ = 0.023). B, ULR. F-values for the main effect of $Time_{deviation}$, and the interaction of VAM x $Time_{deviation}$ were significant at the 0.01, and 0.10 levels, respectively. C, LAR. F-values for the main effects of $Time_{linear}$, $[P]_{linear}$ (inset), and $[P]_{deviation}$ were significant at the 0.01, 0.01, and 0.05 levels, respectively.


A
0.25
0.15
0.05
RGR (g/g/d)
0.14
0.13
0.12
0.0 2.5 5.0
[P] mM P
B
2.8
1.8
0.8
ULR (mg/cm /d)
C
0.11
0.09
0.07
0.05
LAR (cm /mg)
0.081
0.077
0.073
0.0 2.5 5.0
[P] mM P
20 25 30 35 40
DAYS AFTER PLANTING

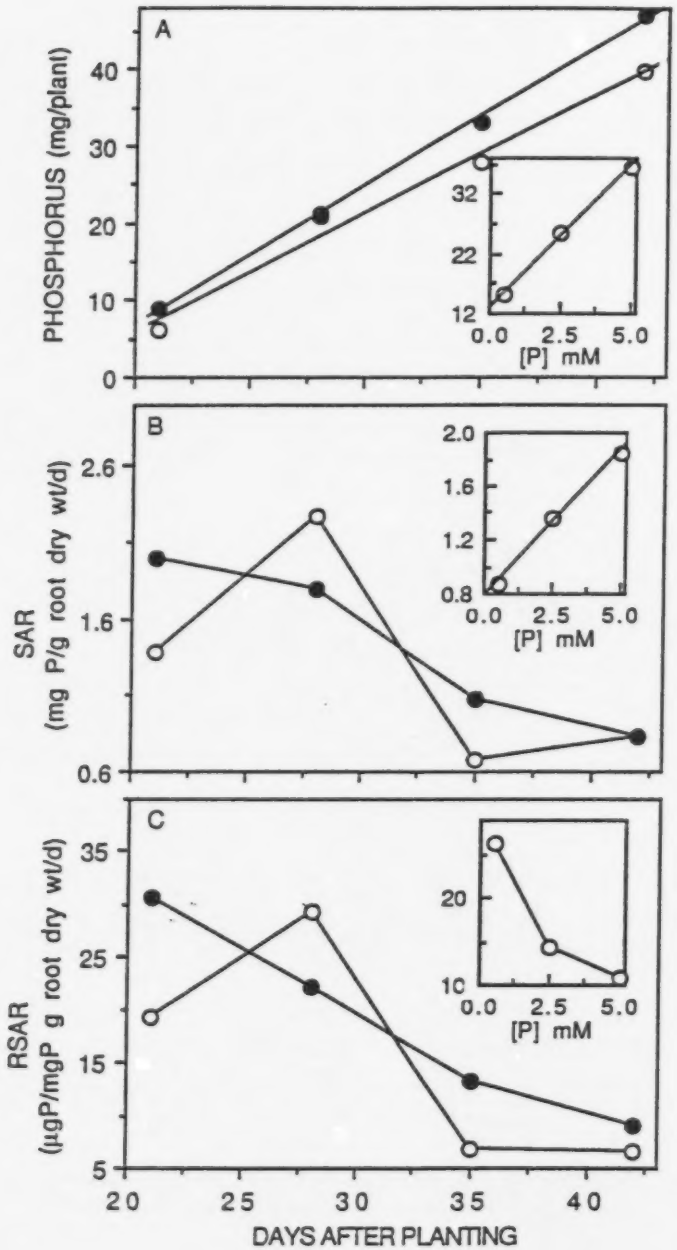
has been attributed to the enhanced flux of photoassimilates to roots to support symbiont growth and respiration (Baas et al., 1989). Under conditions in which the VAM symbiosis significantly alleviates P-deficiency stress, growth may still be stimulated by an increase in LAR (Baas et al., 1989; McArthur and Knowles, 1993). In terms of these growth indices, lack of a clear response to the VAM symbiosis in this study may relate to the somewhat higher plant tissue-P concentrations (see below) and the slower rate of root colonization (Abbott and Robson, 1982; Manjunath and Habte, 1988).

In our study, both ULR and LAR decreased over the harvest interval and thus, defined the decline in RGR with time; however, only LAR was restricted by low abiotic P supply (Fig. 4C, inset). This latter finding indicates that the net assimilatory capacity of leaf biomass (ULR) was not significantly impaired by inadequate P nutrition. Instead, RGR of plants was primarily limited by a restricted ability to develop sufficient photosynthetic surface area (Fredeen and Terry, 1988; Baas et al., 1989).

Whole-plant P accumulation increased linearly over the harvest interval and moreover, was dependent upon both P nutrition and the VAM symbiosis (Fig. 5A and inset). Low-P NM plants had 63% less P by 21 DAP than high-P NM plants. Plant P content increased linearly with abiotic P supply (total P = 12.9 + 4.8[P], r = 0.99) such that, by 42 DAP, low-P and mid-P plants had 62 and 34% less P than high-P plants. VAM plants exhibited a higher rate of P accumulation than NM plants (VAM x $Time_{linear}$, $P < 0.05$) and, as a result, at 35 and 42 DAP, VAM plants had 18% more P than NM plants (Fig. 5A). Greater P accumulation with increasing abiotic P supply was also associated with a linear increase in SAR (SAR = 0.78 + 0.22 [P], r = 0.99) for P (Fig. 5B, inset). The SAR declined with time for both NM and VAM plants (VAM x $Time_{deviation}$, $P < 0.01$) and was similar to the trend for RGR. Similar to the effects on RGR, SAR was marginally greater for NM than VAM plants at 28 DAP ($LSD_{0.10}$ = 0.35 mg P g^{-1} root dry weight d^{-1}). However, VAM plants did have a higher SAR at 21 and 35 DAP, and this is likely the primary reason for the greater P and dry mass accumulation for VAM plants than for NM plants overall. The relative difference in SAR between VAM and NM plants was less than that reported for either *Phaseolus* (Pacovsky et al., 1991) or *Plantago* (Baas et al., 1989); however, the lowest SAR in our study was substantially higher than that of even high-P VAM plants in the bean or the *Plantago* study. Hence, the potato plants in our study apparently had greater access to P than plants in the other studies, although some degree of P deficiency was still apparent.

Although low-P plants accumulated less P than high-P plants and had a smaller root dry mass, a much higher proportion of available P was accounted for in low-P plants than high-P plants (data not shown), and low-P plants demonstrated a relatively high

Figure IV-5. Main effect of time on plant P-status and indices of P uptake for NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. A, whole-plant P content. F-values for the main effects of VAM, $Time_{linear}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{linear}$ were significant at the 0.01, 0.01, 0.01, and 0.05 levels, respectively ($LSD_{0.05}$ = 3.5 mg P). B, specific absorption rate (SAR). F-values for the main effects of $Time_{deviation}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{deviation}$ were significant at the 0.01, 0.01, and 0.01 levels, respectively. C, relative specific absorption rate (RSAR). F-values for the main effects of VAM, $Time_{deviation}$, $[P]_{deviation}$ (inset), and the interaction of VAM x $Time_{deviation}$ were significant at the 0.10, 0.01, 0.05, and 0.01 levels, respectively.


A
PHOSPHORUS (mg/plant)
0
10
20
30
40
32
22
12
0.0
2.5
5.0
[P] mM
B
SAR
(mg P/g root dry wt/d)
0.6
1.6
2.6
2.0
1.6
1.2
0.8
0.0
2.5
5.0
[P] mM
C
RSAR
(µgP/mgP g root dry wt/d)
5
15
25
35
20
10
0.0
2.5
5.0
[P] mM
20
25
30
35
40
DAYS AFTER PLANTING

rate of P acquistion, considering the amount of P available (Fig. 5C, inset). Hence, the RSAR of low-P plants was 2.5-fold greater than that of high-P plants. Similar to SAR, RSAR declined with time, and although NM plants had a higher RSAR at 28 DAP than VAM plants ($LSD_{0.10}$ = 6.3 μg P mg^{-1} P-available g^{-1} root dry weight d^{-1}), VAM plants had a marginally greater RSAR overall than NM plants ($P < 0.10$). Since the actual rate of whole-plant removal of P from soil is largely determined by the amount of absorptive surface (Newman and Andrews 1973), our results suggest that P-deficiency stress induced physiological changes that favored a higher efficiency of nutrient capture by roots. A 'Phosphate starvation inducible rescue system' (Goldstein et al., 1989), which might account for differences in our study between low-P and high-P plants in relation to efficiency of P acquisition, has been described for a number of plants, including potato (Cogliatti and Clarkson, 1983; Goldstein et al., 1988; Lefebvre et al., 1990). Since VAM roots appeared more efficient at P acquisition than NM roots in our study, and as host compatibility to the symbiont was inversely related to abiotic P supply, the VAM symbiosis could be considered as one component of such a rescue system (McArthur and Knowles, 1992 and 1993).

Shoot and root P-concentration declined with time for NM and VAM plants, indicating that P uptake could not match dry matter accumulation (Fig. 6A and 6B). Shoot-P (mg g^{-1} dry weight) was marginally higher for low-P VAM plants than for low-P NM plants ($P < 0.10$), however, increasing abiotic P supply negated this VAM effect (Fig. 6A, inset). In contrast, a highly significant main effect of VAM was evident for root-P, demonstrating an enhanced level of P in roots as a result of symbiosis, regardless of abiotic P supply (Fig. 6B and inset). Moreover, a highly significant ($P < 0.01$) VAM x $Time_{linear}$ interaction indicated that the P concentration of VAM roots declined more slowly than that of NM roots, in spite of more rapid root growth (Fig. 2, insets). Thus, the diference in concentration of root P between NM and VAM plants increased with time. Since some P is stored within intraradical structures, however, the actual contribution of the VAM symbiosis to P ststus of plant root cells is not known (Smith and Gianinazzi-Pearson, 1988; White and Brown, 1979).

Changes to the plant's general mineral physiology in response to differential P nutrition or to the VAM symbiosis were evident through analysis for mineral nutrients other than P. In this regard, the concentration of shoot-K (Fig. 7A) at different harvest dates was significantly higher for VAM plants than for NM plants, except at 28 DAP ($LSD_{0.10}$ = 2.1 mg g^{-1} dry weight). A significant VAM x P_{linear} interaction, however, demonstrated that, while shoot-K concentration was higher for VAM plants than for NM plants under low-P conditions, differences were not significant with high abiotic P

Figure IV-6. Time course of P concentrations for shoots and roots of NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. A, shoot-P. F-values for the main effects of $Time_{linear}$, $[P]_{linear}$, and the interactions of VAM x $Time_{quadratic}$, VAM x $[P]_{deviation}$ (inset) were significant at the 0.01, 0.01, 0.05, and 0.10 levels, respectively. B, root-P. F-values for the main effects of VAM, $Time_{linear}$, $[P]_{linear}$, $[P]_{deviation}$ (inset), and the interaction of VAM x $Time_{linear}$, were significant at the 0.01, 0.01, 0.01, 0.01, and 0.01 levels, respectively.

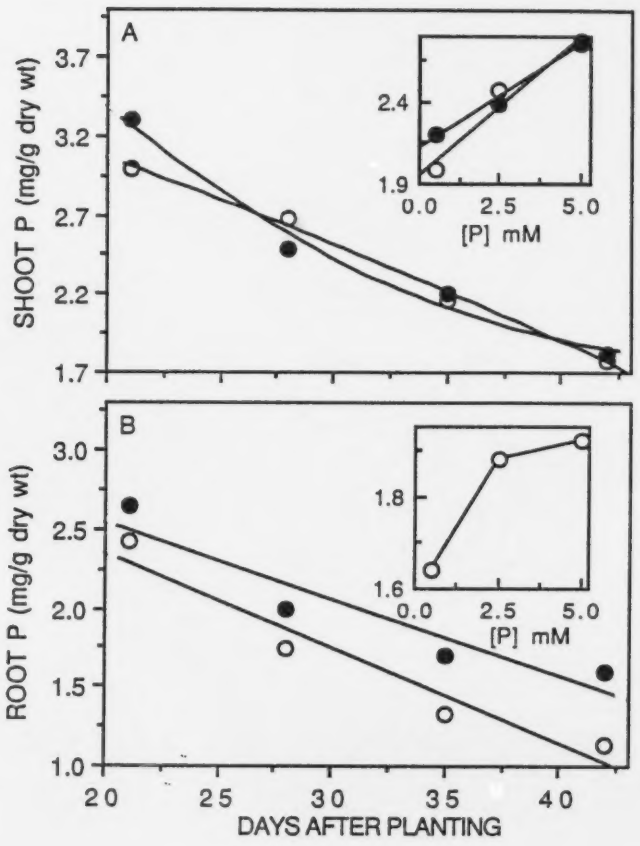
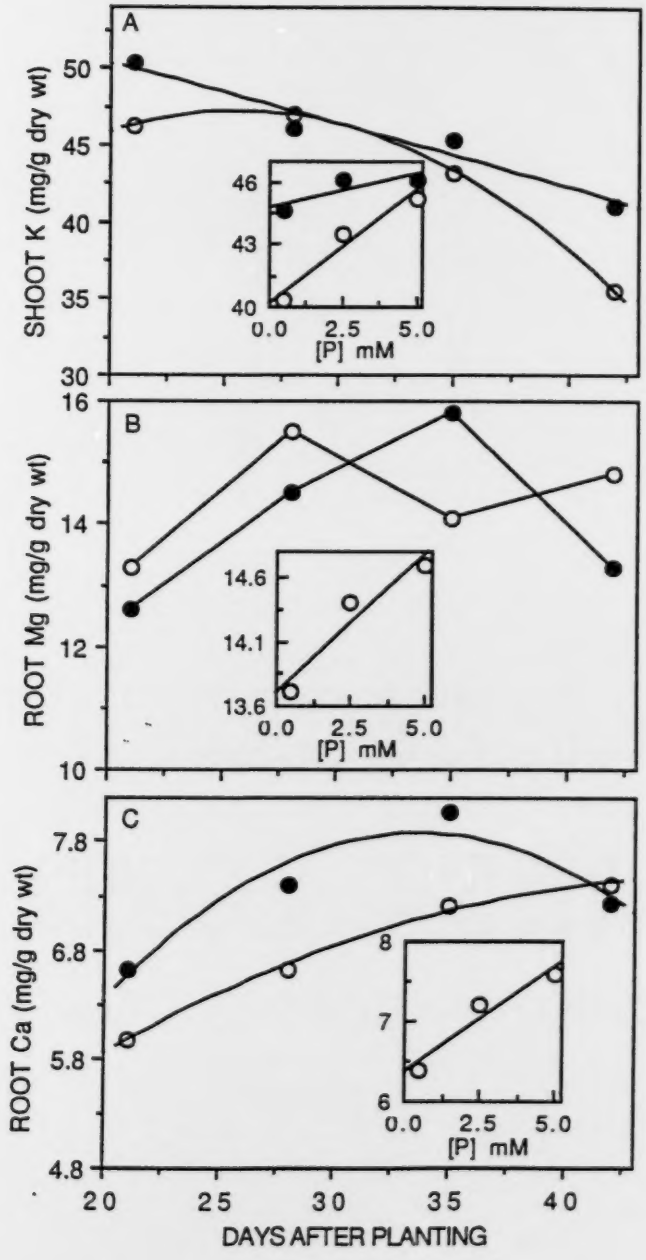

A
SHOOT P (mg/g dry wt)
3.7
3.2
2.7
2.2
1.7
2.4
1.9
0.0
2.5
5.0
[P] mM
B
ROOT P (mg/g dry wt)
3.0
2.5
2.0
1.5
1.0
1.8
1.6
0.0
2.5
5.0
[P] mM
20
25
30
35
40
DAYS AFTER PLANTING

Figure IV-7. Time course of K concentration for shoots, and Mg and Ca concentrations for roots of NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. A, shoot-K. F-values for the main effects of VAM, $Time_{linear}$, $Time_{quadratic}$, $[P]_{linear}$, and the interactions of VAM x $Time_{quadratic}$ and VAM x $[P]_{linear}$ (inset) were significant at the 0.01, 0.01, 0.01, 0.01, 0.01, and 0.05 levels, respectively. B, root-Mg. F-values for the main effects of $Time_{quadratic}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{deviation}$ were significant at the 0.01, 0.10, and 0.01 levels, respectively. C, root-Ca. F-values for the main effects of VAM, $Time_{quadratic}$, $[P]_{linear}$ (inset) were significant at the 0.05, 0.05, and 0.01 levels, respectively.


A
SHOOT K (mg/g dry wt)
50
45
40
35
30
46
43
40
0.0
2.5
5.0
[P] mM
B
ROOT Mg (mg/g dry wt)
16
14
12
10
14.6
14.1
13.6
0.0
2.5
5.0
[P] mM
C
ROOT Ca (mg/g dry wt)
7.8
6.8
5.8
4.8
8
7
6
0.0
2.5
5.0
[P] mM
20
25
30
35
40
DAYS AFTER PLANTING

supply (Fig. 7A, inset). The P-dependent VAM influence on shoot-K concentration indicated that the symbiotic enhancement of K uptake was associated with a VAM influence on plant P accumulation. This suggestion appears to be corroborated by the parallel trend for shoot-P concentration (Fig. 6A, inset). It is of interest to note that K is a key element required for cell extension and osmoregulation (Marschner 1986) and hence, the similarity between the interactive effects of abiotic P supply and VAM infection on shoot-K and shoot-P, and that on the development of total leaf area may be of physiological relevance. The concentration of K in roots was less than half that in shoots and was not altered by either VAM infection or P supply (data not shown).

Unlike K, roots maintained a concentration of Mg that was 1.5-fold that of shoots (data not shown) and moreover, treatment effects were not significant for shoots, but instead only for roots (Fig. 7B). Root-Mg (mg g^{-1} dry weight) increased linearly with abiotic P supply (Fig. 7B, inset); however, no clear effect by the VAM infection was evident. Shoot-Ca (mg g^{-1} dry weight) was marginally higher than that for root-Ca and again, as no clear influence for either VAM infection or P nutrition was evident on shoot-Ca, this data was omitted. Root-Ca concentration increased over the harvest interval, and was an average 8% higher for VAM plants than for NM plants (Fig. 7C). Moreover, root-Ca concentration was linearly related to abiotic P supply (Fig. 7C, inset).

While raising the P nutrition for plants enhanced the concentration of Mg and Ca in roots, no such effect was observed in shoots, in contrast to the findings of Skinner and Matthews for grape (1990). Thus, our results indicate that if root transport of these cations to the shoot was inhibited in our study, it was not due to P deficiency. On the other hand, it is well known that alleviation of P-deficiency stress can improve mineral nutrient uptake generally (Lambert et al., 1979; Lee, 1982). Hence, the higher Mg and Ca concentrations in roots of high-P plants may reflect greater cation uptake for charge-balancing of P, and subsequent vacuolar storage of these cations (Clarkson, 1985). In this regard, we observed the accumulation of calcium oxalate crystals in potato roots in our study (unpublished observations). Higher levels of Ca in response to VAM infection may similarly be related to P nutrition; however, Ca in VAM roots may also relate to charge-balancing of polyphosphates found within intraradical hyphae of the symbiont (White and Brown, 1979).

Shoot and root Cu concentrations (μg g^{-1} dry weight) declined over the harvest interval, averaging 23 μg g^{-1} dry weight, and no distinct influence of either P nutrition or the VAM symbiosis was evident (data not shown). The absence of an effect by P nutrition or the VAM symbiosis on Cu levels in plants appears unusual in that a lower

plant concentration in response to high abiotic P supply, or for NM *versus* VAM plants, has often been observed (Olsen, 1972; Manjunath and Habte, 1988). The lack of treatment effects on tissue-Cu levels in our study may reflect a high availability of Cu to roots due to our application rate (see materials and methods) in conjunction with our growth-medium (predominately sand).

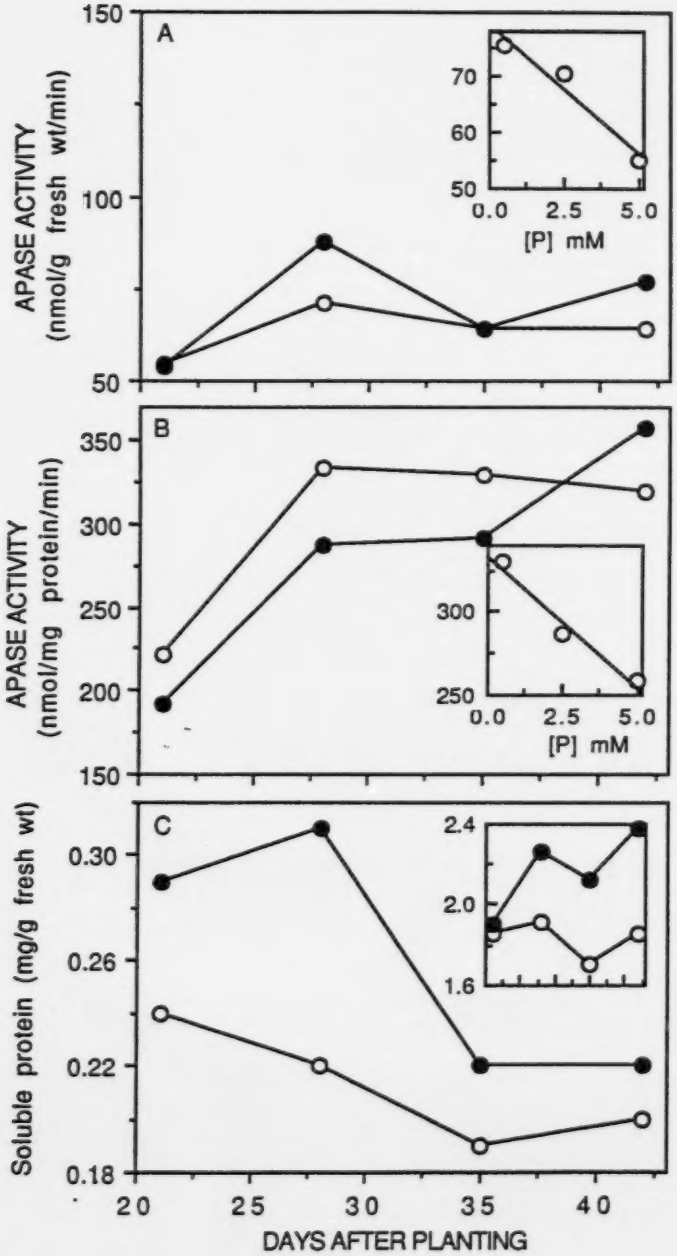
Root Apase Activity

Two major inducible Apase isozymes have been previously demonstrated: one localized in the cell vacuole and another in the cell wall space (Duff et al., 1991; Goldstein et al., 1989). Hence, the activities of both extracellular and total Apases of potato roots were examined concurrently with growth and mineral nutrient analyses to characterize alterations in activity and isozymes in response to P-deficiency stress and VAM infection.

Extracellular Apase activity in root leachates was inversely related to increasing abiotic P supply on a fresh weight (nmol P $g^{-1}min^{-1}$ = 79 - 4.6[P], r = -0.96) and protein basis (nmol P $mg^{-1}min^{-1}$ = 333 - 15.8[P], r = -0.98) (Fig. 8A and 8B, insets). Moreover, extracellular Apase activity on a fresh weight basis (Fig. 8A) was significantly enhanced by the VAM symbiosis, although the significant ($P < 0.05$) VAM x $Time_{deviation}$ interaction showed that differences were only evident at 28 and 42 DAP ($LSD_{0.05}$ = 12.1 nmol g^{-1} fresh weight min^{-1}). This root response to *G. fasciculatum* was similar to those of wheat and onion roots inoculated with *G. geosporum and G. mosseae* (Dodd et al., 1987). For potato roots, however, the greater activity for VAM roots was accompanied by a 20% enhancement of total protein in root leachates in response to VAM infection (Fig. 8C), as a main effect of VAM on extracellular Apase activity on a milligrams protein basis was not observed (Fig. 8B). In fact, a significant VAM x $Time_{quadratic}$ interaction indicated that extracellular activity for VAM roots was initially lower and increased above that of NM roots only late in this study. No influence of abiotic P supply on protein level in either leachates or roots was evident (data not shown).

The activity of Apase from extracts of roots on a fresh weight (nmol P $g^{-1}min^{-1}$ = 1239 - 50.4[P], r = -0.98) or protein (nmol P $mg^{-1}min^{-1}$ = 661 - 35.5[P], r = -0.95) basis was also inversely related to abiotic P supply ($P < 0.01$, data not shown). In contrast to extracellular Apase activity, however, the VAM symbiosis did not influence activity on a fresh weight basis, even though the total protein concentration of VAM roots was an average 18% greater ($P < 0.01$) than that of NM roots (Fig. 8C, inset). In fact, comparison on a protein basis showed that Apase activity of VAM roots

Figure IV-8. Time course of extracellular acid phosphatase (Apase) activity and protein concentration from NM (○) and VAM (●) potato plants grown with 0.5, 2.5, or 5.0 mM P. For extracellular Apase activity and proteins, roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 25 mM NaCl at 25°C for 2.5 h. A, Apase on a grams fresh weight basis. F-values for the main effects of VAM, $Time_{deviation}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{deviation}$ were significant at the 0.05, 0.01, 0.01, and 0.05 levels, respectively. B, Apase on a milligrams protein basis. F-values for the main effects of $Time_{deviation}$, $[P]_{linear}$ (inset), and the interaction of VAM x $Time_{quadratic}$, were significant at the 0.05, 0.01, and 0.10 levels, respectively. C, extracellular root protein concentration. F-values for the main effects of VAM, and $Time_{deviation}$ were significant at the 0.05 level. Inset: Time course of protein concentration of root extracts, F-values for the main effect of VAM and the interaction of VAM x $Time_{linear}$ were significant at the 0.01 and 0.05 levels, respectively.

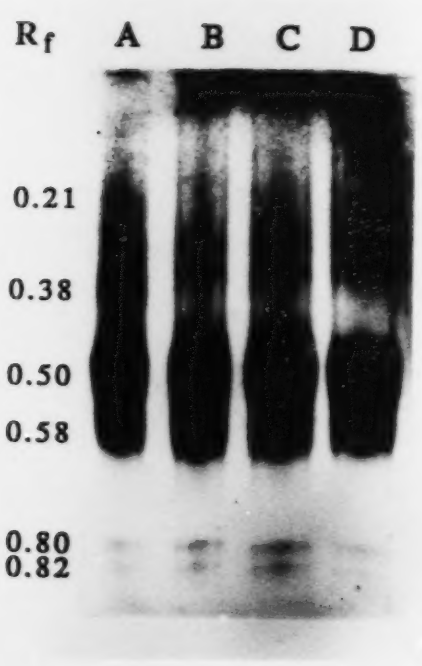

A
APASE ACTIVITY
(nmol/g fresh wt/min)
150
100
50
70
60
50
0.0
2.5
5.0
[P] mM
B
APASE ACTIVITY
(nmol/mg protein/min)
350
300
250
200
150
300
250
0.0
2.5
5.0
[P] mM
C
Soluble protein (mg/g fresh wt)
0.30
0.26
0.22
0.18
2.4
2.0
1.6
20
25
30
35
40
DAYS AFTER PLANTING

was marginally lower than that of NM roots (534 *versus* 600 nmol P mg^{-1}protein min^{-1}). These results are consistent with our previous findings (McArthur and Knowles 1993), and those of Gianinazzi-Pearson and Gianinazzi (1976) and Capaccio and Callow (1982), who both examined total Apase activity of onion roots infected by *G. mosseae* although, only on a fresh weight basis.

Proteins were concentrated and then separated by native PAGE to compare isozymes for soluble extracellular (Fig. 9A) and total Apases (not shown) from roots of NM plants and VAM plants, grown with either low or high abiotic P supply. For both extracellular and total Apases, two distinct minor bands with an R_f of 0.80 and 0.82, plus an intensely-stained band with an R_f of 0.50 and a more diffuse band with an R_f of 0.58 were observed at all harvest dates and regardless of treatment. Relatively more of the latter isozyme was observed in leachates than for extracts and hence, it appeared likely to be an extracellular protein. A secreted Apase isozyme from mustard cells, with an R_f intermediate to those from mustard cell extracts, has also been demonstrated (Lefebvre et al., 1990). In addition, both secreted and intracellular Apase isozymes were evident in these mustard cells when grown over a wide range of P levels, as in our study. Various extracellular isozymes with R_f values ranging between 0.21 and 0.38 were also observed for potato roots, however, these had very low levels of activity staining and were not present at all four harvest dates (not shown). No qualitative or quantitative differences due to the VAM symbiosis or P nutrition were discernible by isozyme analysis.

The pattern of isozyme separation of extracellular Apases from potato roots was also somewhat similar to that demonstrated for root extracts of NM and VAM bean (Pacovsky et al., 1991) and for NM and VAM onion plants (Gianinazzi-Pearson and Gianinazzi, 1976). Moreover, our findings are consistent with those on bean and onion plants in that no new Apase isozymes were evident in response to the VAM symbiosis. In contrast to the findings of Pacovsky et al (1991), however, we were not able to detect enhanced activity staining for any particular isozyme in response to the VAM symbiosis. Differences between host plants, and difficulties in making quantitative comparisons of enzyme activity with native PAGE are likely responsible for this inconsistency. On the other hand, we note that mycorrhizal bean plants at 28 DAP had a very low level (12.5%) of VAM infection and moreover, showed no response to the VAM symbiosis, in terms of the amount of P accumulated at 28 DAP. Hence, the VAM enhanced activity staining observed for one of the bean Apase isozymes by Pacovsky et al (1991) was not likely one associated with symbiotic nutrient exchange, in any event.

Figure IV-9. Acid phosphatase (Apase) activity stain of native PAGE for extracellular proteins. Extracellular Apase activity was obtained from leachates at 28 DAP from roots excised from NM (lane A and C) and VAM (lane B and D) potato plants grown with 0.5 (lane A and B) or 5.0 (lanes C and D) mM P. Approximately 0.1 µg of protein was loaded for all treatments. To obtain leachates, roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 25 mM NaCl at 25°C for 2.5 h.


R_f
A
B
C
D
0.21
0.38
0.50
0.58
0.80
0.82

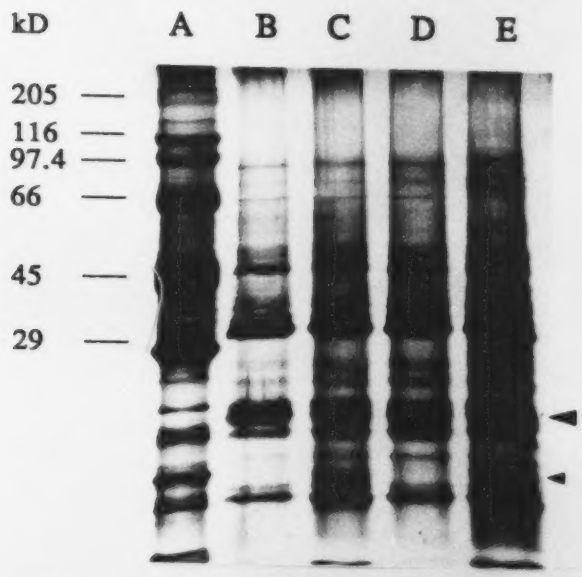
Profile of Soluble Root Proteins

Soluble, extracellular proteins from leachates were characterized by silver staining after separation by SDS-PAGE. Extracellular proteins from low-P and high-P did not differ over the four harvest dates (not shown); however, at 28 DAP (Fig. 10A) and thereafter, a unique protein of approximately 10 kD (small arrow) was present in leachates of low-P VAM roots (lane C). A similar protein band was evident for high-P VAM plants only at 35 and 42 DAP (not shown).

Coomassie-stained gels of root extracts showed a protein separation pattern similar to that of leachates; however, differences in the types of bands present and the relative staining of shared bands were observed. These differences are likely attributable to the relatively higher proportion of extracellular proteins in leachates in comparison to those from root extracts. For example, the pronounced bands between 12 to 16 kD (arrowhead) in gels for leachates (Fig. 10A) were almost absent in gels for root extracts (Fig. 10B). Similar to the gels for extracellular proteins, however, no difference in protein composition due to differential P nutrition was observed. While the 10 kD band was not apparent in root extracts, a protein of approximately 9 kD (small arrow) and also found in leachates was specifically enhanced only for VAM roots (lanes C and H), and this occurred within the same time frame as the 10 kD band for leachates. In an opposite trend, the presence of a 43 kD protein (large arrow) of plant origin was noticeably diminished in extracts from low-P VAM roots at 28 DAP (lane C) and thereafter (not shown), and also for high-P VAM roots at 35 (not shown) and 42 DAP (lane H). Pacovsky (1989), reporting on interactions between soybean and *G. fasciculatum*, also observed the appearance in VAM root extracts of a number of smaller molecular weight proteins (15 to 30 kD) or 'endomycorrhizins' and noted a depletion of some high molecular weight proteins (> 40 kD, Fig. 1, pp 351). Although the origin of the low molecular weight proteins of VAM roots has not been determined for either soybean or potato, and since their appearance was associated with a diminished level of higher molecular weight plant proteins, there is the possibility that such 'endomycorrhizins' as observed in roots of potato or soybeans may result from a fungal stimulation of degradation of some specific plant proteins. It has been demonstrated in this and other studies that protein content and composition of roots are substantially altered by infection with a VAM symbiont (Cox and Tinker, 1976; McArthur and Knowles, 1993; Pacovsky, 1989). Further characterization of this VAM influence on protein metabolism merits consideration, as such information should provide greater insight into the mechanisms by which nutrient exchange and compatibility operate.

Figure IV-10. Protein pattern of soluble extracellular (A) and total (B) proteins from roots of NM and VAM potato plants grown with 0.5 or 5.0 mM P. A, silver-stained SDS-PAGE of extracellular proteins of low-P NM (lane B), low-P VAM (lane C), high-P NM (lane D) and high-P VAM (lane E) plants at 28 DAP. Molecular weight markers are in lane A. To obtain extracellular protein, leachates were collected from roots that had been incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 25 mM NaCl at 25°C for 2.5 h. Approximately 2 or 25 µg protein were loaded for extracellular or total root proteins, respectively. A similar amount of protein was loaded for each treatment within each gel. B, coomassie-stained SDS-PAGE of total proteins of low-P NM (lane B), low-P VAM (lane C), high-P NM (lane D) and high-P VAM (lane E) plants at 28 DAP and high-P NM (lane G) and high-P VAM (lane H) plants at 42 DAP. Molecular weight markers are in lane A and F.

A



B

In conclusion, it was clearly evident by characterizing plant growth and P content that, as early as 21 DAP, plants receiving low abiotic P supply had a lower growth potential than those with a greater level of P nutrition (Fig. 2 and Fig. 5). Over the harvest interval, this difference in growth potential was manifest by reductions in biomass accumulation and mineral nutrient status (Fig. 3 and inset, Fig. 4), and by an altered dry matter partitioning (Table I) for low-P plants compared to high-P plants. Moreover, changes to plant physiology, such as an enhanced compatibility to the VAM symbiosis, RSAR for P, and Apase activity of roots, in response to low P nutrition were observed (Fig. 1, Fig. 5C inset, Fig. 8B and inset). These results are consistent with observations of others on the effects of P-deficiency stress (Goldstein et al., 1988; Lefebvre et al., 1990) and moreover, support our previous suggestion that low abiotic P supply had altered the physiology of potato plants to improve the efficiency of P acquisition and utilization (McArthur and Knowles, 1992). In this regard, we note that, under the more severe conditions of P-deficiency stress, the VAM symbiosis was by far the most efficient means by which plants could obtain P for continued growth.

Although Apases have been implicated in the process of nutrient exchange and hence, in the level of compatibility between symbionts, our results do not add support to this hypothesis. Neither new isozymes nor enhanced levels of any existing isozymes were evident in response to VAM infection by activity stains of extracellular and intracellular Apases (Fig. 9). Previously, we had demonstrated a large increase in root microsomal ATPase activity in response to the VAM symbiosis, likely reflecting an arbuscular stimulation of root plasma membrane synthesis (McArthur and Knowles, 1993). It is also of interest that the specific Apase activity associated with the microsomal membranes was also decreased, both by high-P and by the VAM symbiosis. The specific activity of soluble Apases in the current study was decreased by the VAM symbiosis and only extracellular Apase was enhanced on a fresh weight basis and furthermore, only on two of four harvest dates (Fig. 8A). Moreover, timing of the enhanced Apase activity did not completely coincide with the symbiotic-enhancement of plant P content (Fig. 5A), nor did it reflect the trend for root infection (Fig. 1). Hence, changes to Apase activity in response to the VAM symbiosis were consistent with those associated with an improved P nutrition, or only in conjunction with a substantially increased protein content of roots (Fig. 8C). While specific changes to Apases by the VAM symbiosis were not evident, changes to protein composition in roots (Fig. 10) indicate an area for further investigation. In this regard, the characterization of proteins which are specifically induced by the VAM symbiosis may further our understanding of the mechanisms involved in nutrient exchange and compatibility.

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Chapter V

Resistance Responses of Potato to VA-Mycorrhizal Fungi Under Varying Abiotic Phosphorus Levels[4]

Introduction

The vesicular-arbuscular mycorrhizal fungi are a small and distinct group of obligate, symbiotic fungi that are capable of association with roots of most terrestrial vascular plants (20). In exchange for photoassimilates, VAM fungi contribute to the plant acquisition of P and other mineral nutrients and, by this means, stimulate the growth of host plants. Although genotypic and environmental factors influence the symbiosis, a prolonged, stable and compatible association between plants and VAM fungi is based primarily upon the transfer of P from the symbiont to a host which has a net P deficit. When available P in the soil solution is non-limiting to plant growth, roots exclude or limit VAM infection. Hence, plants appear capable of exerting substantial control in their interactions with these symbionts, although the physiological, biochemical, and genetic bases for host control are not well understood (29, 32).

Mechanisms by which plants restrict VAM growth appear to involve nutritional or non-nutritional factors as their basis, and may include both (32). The ability of plants to regulate partitioning of carbohydrates appears to play a central role in their control of the VAM infection process. Evidence for this comes from experiments on how plant developmental stages, nutritional components (including P), and the VAM symbiosis alter source to sink relations, root exudation, and photosynthetic activity (22, 29). On the other hand, recent investigations into the influence of phenolic compounds on hyphal growth and root colonization implicate several secondary metabolites (31) as promoters of VAM growth and development. Insight into this area of research can be gleaned from investigations of legume:rhizobium interactions, where specific metabolites of the phenylpropanoid pathway play a key role in the establishment of the symbiosis. Thus, the observed suppression of fungal colonization of roots when the external, i.e. abiotic, P-supply increases may be due to the plant somehow limiting the availability of photoassimilates needed for fungal nutrition, or specific regulatory signals from the plant which directly control symbiont growth.

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[4] A version of this chapter has been accepted for publication. McArthur & Knowles 1992. Plant Physiology. In press.

Also meriting consideration is the observation that the invasive growth of VAM fungi during infection is similar to that in many host:pathogen interactions (3, 16). VAM infection hyphae can induce papilla-like thickenings in epidermal cells, and the activities of cell wall-bound POX's (involved in lignin formation) and chitinases are enhanced during the early stages of infection by VAM fungi (33, 34). Such observations are consistent with those of a pathogen-induced defense response by plants (37). In this regard, roles for phenolic metabolism and lignin production in the resistance responses by plants to various types of mycorrhizae have been suggested (3).

Many resistance responses of plants to parasites involve the ethylene and phenylpropanoid biosynthetic pathways (13, 19, 37). Activation of these pathways can stimulate the generation of fungitoxic compounds (e.g., oxidized polyphenols from increased PPO activity) and create lignin barriers (e.g., the actions of extracellular POX on ferulic acid) which may physically impede infection (37). Involvement of the plant defense response in host:VAM fungi interactions appears feasible, since, in addition to the above observations, VAM infection increases the level of phenolic compounds in roots (24), and moreover, ethylene (Ethrel) treatment of roots or shoots inhibits VAM infection (4, 27). A coincident increase in phytoalexins has been associated with a lower level of infection after ethylene treatment (27).

This paper describes morphological and biochemical details of interactions between VAM fungi and potato (*Solanum tuberosum* L. cv Russet Burbank) roots after infection. The extent to which the plant's defense responses are involved in P-induced resistance to VAM fungi (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) is central to our investigation. Our objectives were to characterize the effects of abiotic P-supply and VAM fungal infection on root ethylene production and phenolic metabolism. The results demonstrate that the root's capacity for ethylene production via ACC_{ox} activity is restricted by VAM infection. In contrast, the activities of other enzymes which have been implicated in plant defense responses, such as PPO and extracellular POX, were unaltered or only mildly stimulated in response to VAM infection. However, as abiotic P-supply increased, the root capacity for ethylene production and extracellular POX activity also increased, while VAM infection of roots declined. The physiological basis for this P-induced resistance to VAM infection, and the paradox that plants cope with in shifting their priorities between P nutrition and defense are discussed.

Materials and Methods

Inocula

Inocula for NM and VAM (*Glomus fasciculatum* [Thaxt. sensu Gerdemann] Gerdemann and Trappe) treatments consisted of soil and roots of clover (*Trifolium repens* L. cv Altaswede) plants grown in an autoclaved sand:soil (v/v 3:1) medium for 90 d. Plants were grown in 2-L pots and were fertilized weekly with 50 mL of a modified (minus P) Hoagland's solution. Roots of NM clover plants were uninfected, while those of VAM plants were 85% infected and had many vesicles. Few mature chlamydospores were present on either the surface of roots or in the soil of the VAM inoculum. To quantify infection, roots were cut into 1-cm lengths and were stored in FAA solution (5% formalin, 5% acetic acid, 45% ethanol) until further processing by clearing and staining (28). Percent infection was assessed using the gridline intersect method (17) with 100 counts per slide, and five (clover) or three (potato [*Solanum tuberosum* L cv Russet Burbank]) slides per plant.

Plant Growth Conditions

Certified potato seed-tubers, taken from a 4°C (95% RH) storage, were surface-sterilized with 1.0% (v/v) sodium hypochlorite (3 min) and rinsed thoroughly with dH_2O. Single-eye seedpieces (5.0 g) were cut from the mid-region of the tubers and rinsed. After air-drying for 1 h, the seedpieces were planted in vermiculite and sprouted in the dark for 10 d at 27°C (95% RH).

Single-sprout seedpieces were blocked for sprout length prior to transplanting onto NM or VAM soil inoculum (100 g f.wt. pot^{-1}) in 20-cm diameter pots (1 seedpiece pot^{-1}). Each pot contained 3.4 kg (air-dry) of an autoclaved soil medium consisting of 6:1 v/v sand:soil (3 µg/g $NaHCO_3$-extractable P). Pots were placed in a growth chamber set at 25/20°C (day/night) with a 16-h photoperiod. A PPFD of 480 µmol $m^{-2}s^{-1}$ was provided by fluorescent and incandescent lights maintained at 25 cm from the shoot tips. Each pot received nutrients as indicated below. For split-root studies, sprouted seedpieces were produced as above and transplanted with half of their roots positioned in a separate uninoculated pot and the other half in a VAM-inoculated pot. Each root-half received half of the total nutrient solution containing 0.5 mM P as described below. In the first week, each pot received 50 mL of nutrient solution three times [(mM): 40, KNO_3; 20, $Ca(NO_3)_2$; 20, $MgSO_4$; µM: 185, H_3BO_3; 36.5, $MnCl_3$; 0.3, $ZnSO_4$; 1.3 $CuSO_4$; 0.065 H_2MoO_4; 2 mg/L Fe-EDDHA (pH 6.0)], then 100 mL with added P (0.5 or 2.5. mM NaH_2PO_4) was given three times per week thereafter.

A second study was completed with sprouted seedpieces produced and treated as in the previous experiment, except that 50 g of soil inoculum from NM or VAM clover plants

was used (VAM roots were 80% infected, with spores absent, but vesicles abundant.). Pots were placed under growth conditions as above, but with a PPFD of 540 μmol $m^{-2}s^{-1}$, and the lights were maintained 14 cm from shoot tips. Essential nutrients were provided as described above.

Experimental Design and Analytical Methods

In the first study, treatments consisted of all four combinations of two levels of P (0.5, 2.5 mM), and two inocula (NM or VAM) in a randomized complete block design (five blocks). Blocks were harvested at 33, 38, 43, 45, and 57 (average 43) DAP for measurement of ethylene production and the apparent ACC_{ox} activity in root systems. Two supplementary experiments were also initiated. In the first, plants were inoculated as above but grown on nutrient solution containing only the 0.5 mM level of P. These plants were harvested at 83 DAP (two blocks) to examine the effect of VAM-root leachate (pooled samples from the 43 DAP study) on root ACC_{ox} activity. In the second, plants grown with their roots divided (+/- inoculum) were harvested at 85 DAP (four blocks) for determination of ACC_{ox} activity.

Roots of intact plants were gently washed in running water, rinsed in cold deionized-dH_2O, and stored briefly (max 30 min) in cold deionized-dH_2O (4°C). The roots were then blotted dry with filter paper, and groups of fine roots from the mid-region of each root system were cut into 1-cm lengths. Excised roots were placed in triplicate 15-mL disposable test tubes (1 g f.wt. $tube^{-1}$) containing 2 mL of 50 mM Mes (Tris) buffer (pH 6.5), with or without 10 mM ACC. Tubes were capped with serum stoppers and incubated at 23°C for up to 7.5 h. Ethylene production by excised roots was measured at 2.5-h intervals by injecting a 1-mL gas sample from each tube into a gas-chromatograph (Hewlett-Packard, model 5890A) equipped with a flame ionization detector. The 1-mL gas samples were replaced with an equal volume of air at each sampling. The remaining shoots, tubers, and roots were lyophilized for further analyses.

In another experiment, treatments consisted of four harvest dates (21, 28, 35, and 42 DAP), three levels of nutrient solution P (0.5, 2.5, and 5.0 mM), and two inocula (NM or VAM) arranged factorially in a randomized complete block design (three blocks). Measurements (in triplicate) of root ACC_{ox} activity were as described above; however, each tube contained 0.5 g f.wt. of roots and 1 mL of buffer containing 10 mM ACC. Roots were incubated for 2.5 h at 25°C. In addition, 15-mL test tubes (in duplicate), each containing 0.5 g f.wt. of roots in 5 mL of 50 mM Mes (Tris) (pH 6.5) buffer with 25 mM NaCl, were simultaneously incubated for measurement of leached phenolics and POX.

Filtrates were collected for the assays, and the roots were lyophilized. The remaining roots for each plant were frozen (-20°C) for later extraction. Phenolics and POX were extracted (mortar and pestle) at 4°C in Mes (Tris) buffer (50 mM; pH 6.5) containing 2.5 mM $MgSO_4$ (1 g f.wt. roots to 10 mL buffer). Extracts were filtered through a double layer of miracloth and centrifuged at 15,000g for 20 min (4°C). Pellets were discarded and supernatants were used for determinations of POX and total soluble phenolics. Protein was assayed by the method of Bensadoun and Weinstein (6) with BSA as a standard.

Phenolics in 1 mL of leachate or 0.5 mL of root extract were assayed by the method of Amerine and Ough (2). Samples were incubated at 25°C in a shaking water bath for 15 min and read at A_{765}. Total phenolics were quantified with 0.5 mM ferulic acid (in 0.5% ethanol) as a standard. For estimation of polyphenol oxidase activity (5), 100 µL of root extract was added to 3 mL of 5 mM DOPA, and the change in A_{475} was followed over a 2-min interval. The linear phase was used to calculate PPO activity, which was expressed as the change in absorbance min^{-1} on a g f.wt. or a mg protein basis. Peroxidase activity was determined at A_{470} (5). A 50-µL aliquot of filtrate (25 µL of root extract) was added to 3 mL of 50 mM sodium acetate buffer (pH 5.5) containing 2.5 mM $MgSO_4$, 1.0 mM $CaCl_2$, 0.03% H_2O_2, and 15 mM guaiacol (made fresh before use). Enzyme activity was expressed as the change in absorbance s^{-1} on a g f.wt. or a mg protein basis.

Microscopy

Washed roots were cut into 3-mm pieces, transferred to phosphate buffer (100 mM; pH 6.8) containing 5% glutaraldehyde and incubated for 2 h at 23°C. The root pieces were rinsed three times with phosphate buffer and then incubated in phosphate buffer containing 2% osmium for 2 h. After three additional rinses with phosphate buffer, roots were plunged into liquid N_2, fractured with a precooled scalpel, and lyophilized overnight. Roots were mounted with double-sided sticky tape on stubs, gold-sputter-coated, and viewed with a Cambridge Stereoscan 150 SEM. Some glutaraldehyde-fixed roots were frozen in liquid N_2 and fractured in an Emitech K1250 SEM Cryosystem. Water was sublimated from the fractured surface of frozen roots for 30 min before examination with the SEM.

Statistical Analyses

Growth and physiological data were subjected to analysis of variance and, where appropriate, sums of squares were partitioned into individual degree of freedom

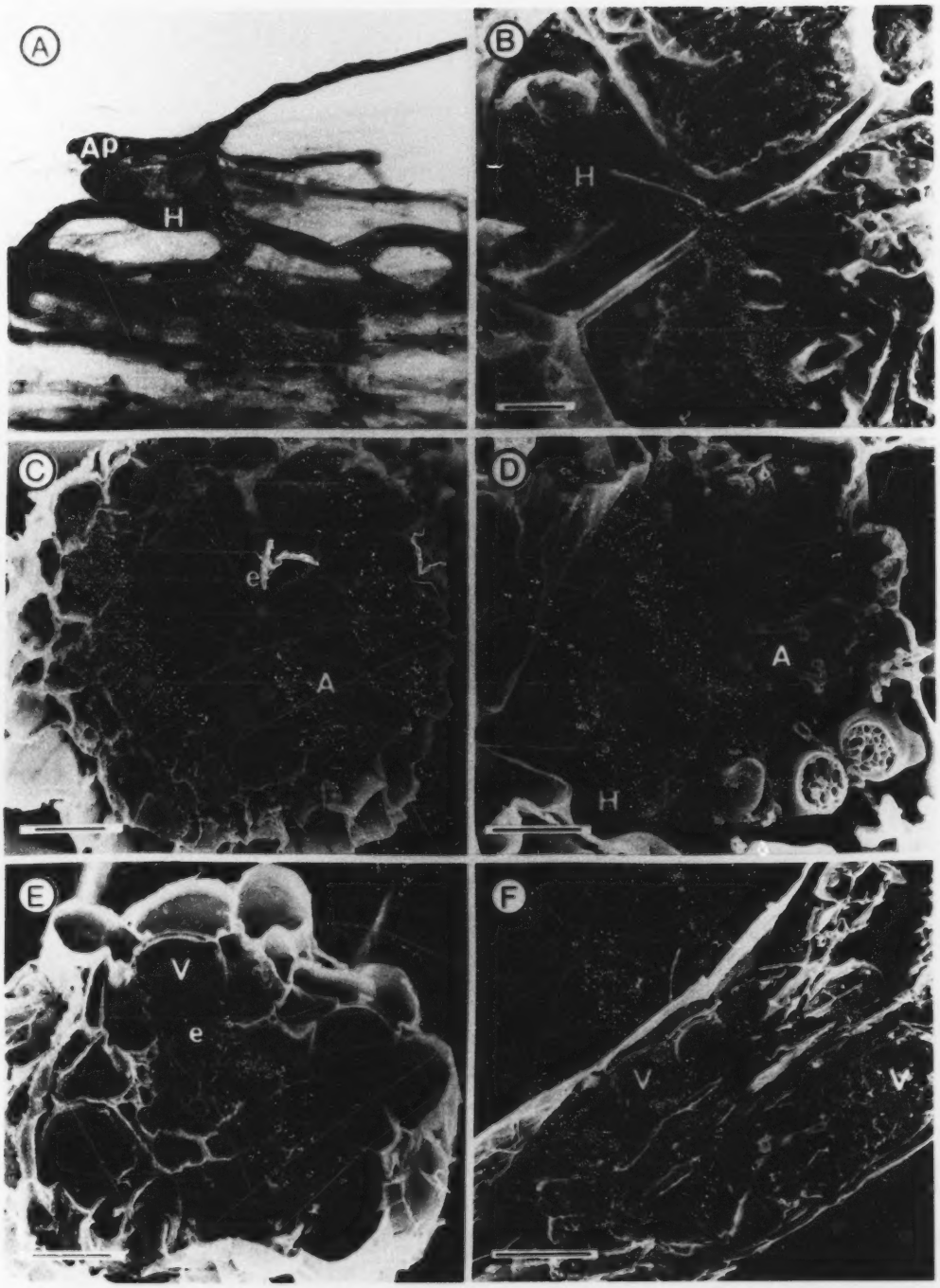
components of both main effects and interactions. Percent-infection data were arcsin transformed to achieve homogeneity of variance prior to ANOVA. Based on the ANOVA results, regression analysis was used to derive polynomial models describing the various relationships.

Results

The invasive nature of VAM fungi in roots and a perspective of the plant:fungus interface are illustrated by the micrographs in Figure 1. Extraradical hyphae adhering to the epidermal surface produced swollen appressoria, while the hypha penetrating through the cell wall constricted to facilitate entry (Fig. 1A). Once through the cell wall, the hypha again attained a swollen appearance before returning to a size more typical of an intracellular hypha (Fig. 1A and B). Hyphal movement through cortical cell walls appeared less difficult and often did not involve visible alterations in hyphal diameter (Fig. 1B). A high proportion of parenchyma cells in the cortical region of potato roots was colonized (Fig. 1C). As a result, many arbuscules interfaced with cells directly adjacent to the endodermis, thus increasing the potential for nutrient interception by the fungus (15). The fine, dichotomous hyphae of the arbuscule filled most of the cell lumen; however, these terminal structures eventually collapsed, while thicker intraradical hyphae continued growth into other cells (Fig. 1D). Although arbuscules and vesicles (Fig. 1E) differ in size and form, both appeared to have a major spatial and physical impact on the infected root cells. Vesicles frequently appeared to fill the cell lumen (Fig. 1E and F), and counts of vesicles in highly colonized roots often exceeded 250 per cm.

In the initial study, vesicles appeared to predominate over arbuscules and other VAM structures, although no formal attempt was made to quantify either. Moreover, high levels of root colonization were evident in VAM roots at 33 DAP, and these levels were maintained thereafter (e.g., 89% and 76% infection for plants grown on 0.5 mM and 2.5 mM P, respectively). Although VAM infection stimulated plant growth at either level of abiotic P-supply (e.g., at 63 DAP, root growth was 2.5 and 3.0 g for VAM plants versus 1.8 and 2.7 g for NM plants for low and high P, respectively; $P < 0.05$), and increased root P concentrations (for low and high P respectively, 1.4 and 1.7 mg g d.wt.$^{-1}$ for VAM plants versus 0.9 and 1.1 mg g d.wt.$^{-1}$ for NM plants; $P <$

Figure V-1. Light and scanning electron micrographs of potato roots infected with the VAM fungus, *G. fasciculatum*. A. An extraradical hypha penetrating the root epidermis via an appressorium (Ap) and producing intracellular hyphae (H) (Magnification x2000). B. Cryostage SEM of a root showing an intracellular hypha (H) penetrating through a cell wall (arrow) (Bar = 10 μm). C. Transverse fracture of a root illustrating the predominance of arbuscules (A) in the cortex, and exclusion of the VAM fungus from the stele by the endodermis (e) (Bar = 40 μm). D. An arbuscule and intracellular hyphae within a cortical cell (Bar = 10 μm). E. Transverse fracture exposing numerous vesicles and showing exclusion of the fungus from the stele by the endodermis (Bar = 40 μm). F. Longitudinal fracture showing adjacent intact vesicles and fractured vesicles (right) (Bar = 100 μm).


A
Ap
H
B
H
C
e
A
D
A
H
E
V
e
F
V

0.01), the high abiotic P-supply had a negative influence on VAM infection ($P < 0.01$). High P nutrition thus somehow altered the host's physiology, rendering the plant more resistant to VAM infection.

Although changes in host compatibility in response to P supply may reflect alterations in many physiological processes (e.g., the flux of nutrients from plant to fungus), the plant's defense response is likely to be a good indicator of the degree of compatibility between host and symbiont (3, 37). Since ethylene biosynthesis by plants is known to be a component of their defense response, the interactive effects of P and VAM infection on ethylene biosynthesis were examined.

For roots without ACC in the incubation buffer, ethylene production from either NM or VAM plants at 43 DAP was relatively low (Fig. 2), and the rate of ethylene production (maximum 0.37 nmol g d.wt.$^{-1}$ h^{-1}) decreased over the 7.5-h incubation period (Fig. 2 inset). However, even at these low levels, VAM-infected roots produced significantly less (26%) ethylene over the incubation interval than did NM roots. Root ethylene biosynthesis was much higher when ACC was included in the buffer, indicating that the probable source of ethylene generating activity was ACC_{ox} (Fig. 3). Consistent with previous findings (12), roots of plants grown with higher levels of P exhibited higher ACC_{ox} activities than those grown with lower P. For plants grown with high P, the inhibitory effect of VAM infection on the ability of roots to convert ACC to ethylene, presumably via ACC_{ox}, was even more pronounced. ACC_{ox} activity of VAM roots was 42% and 75% lower than that from NM roots of plants grown under low and high levels of P, respectively (Fig. 3 inset), reflecting a highly significant [P] x VAM interaction ($P < 0.01$). In this regard, the inhibitory effect of VAM infection on the ability of roots to produce ethylene is somewhat at odds with the potential enhancement of P nutrition for plants provided by the fungus. The P concentration in roots of VAM-infected plants (1.8 mg g d.wt.$^{-1}$) was 50% greater (averaged over P levels and time) than roots of NM plants (1.2 mg g d.wt.$^{-1}$) in this study. Hence, VAM infection somehow interferes with the root's capacity to produce ethylene over and above the main effects of P.

Roots of plants harvested as late as 85 DAP displayed the inhibitory effect of VAM infection on ACC_{ox} activity and thus ethylene production (Fig. 4 and inset). By utilizing plants grown with their roots split into two compartments (+/- inoculum), we demonstrated that the inhibition (82%) of ACC_{ox} activity was localized to the VAM-infected side of the root system. Thus, the uninoculated half of roots from a VAM plant had an ACC_{ox} activity comparable to that of the uninoculated roots of a NM

Figure V-2. Ethylene production (ACC_{ox} activity) by excised roots from 43-day-old potato plants grown with 0.5 mM (○, ●) or 2.5 mM P (□, ■) and inoculated with NM (○, □) or VAM (*G. fasciculatum*) (●, ■) clover inoculum. Roots were incubated in Mes (Tris) buffer (pH 6.5) at 23°C for 7.5 h. F-values for the main effects of VAM, $Time_{linear}$ and $Time_{quadratic}$ were significant at the 0.05, 0.01 and 0.01 levels, respectively. Inset: change in ethylene production rate for NM (○) and VAM (●) roots during incubation.

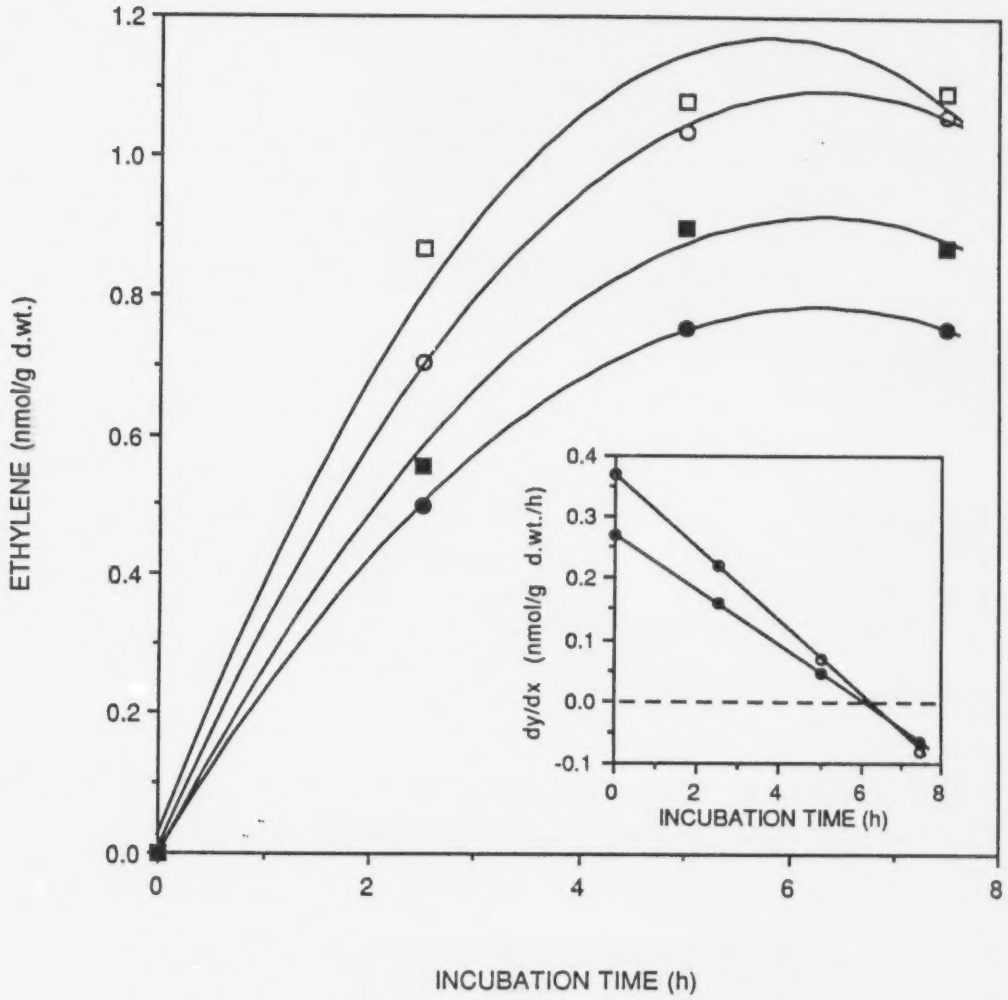

ETHYLENE (nmol/g d.wt.)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
INCUBATION TIME (h)
dy/dx (nmol/g d.wt./h)
0.4
0.3
0.2
0.1
0.0
-0.1
0
2
4
6
8
INCUBATION TIME (h)

Figure V-3. Ethylene production (ACC_{ox} activity) by excised roots from 43-day-old potato plants grown with 0.5 mM (○, ●) or 2.5 mM P (□, ■) and inoculated with NM (○, □) or VAM (*G. fasciculatum*) (●, ■) clover inoculum. Roots were incubated in Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 7.5 h. F-values for the main effects of [P], VAM, $Time_{linear}$ and the interactions [P] x VAM, [P] x $Time_{linear}$, VAM x $Time_{linear}$, and [P] x VAM x $Time_{linear}$ were all significant at the 0.01 level. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.

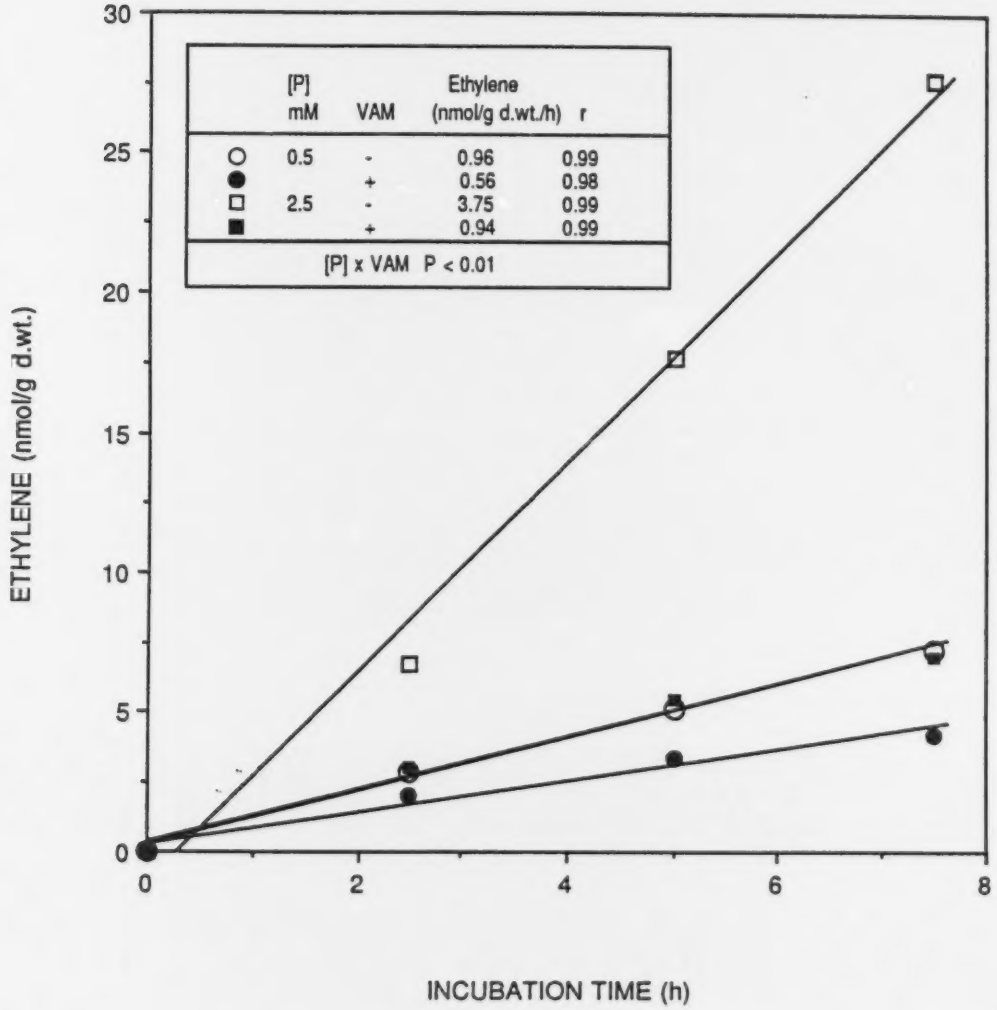
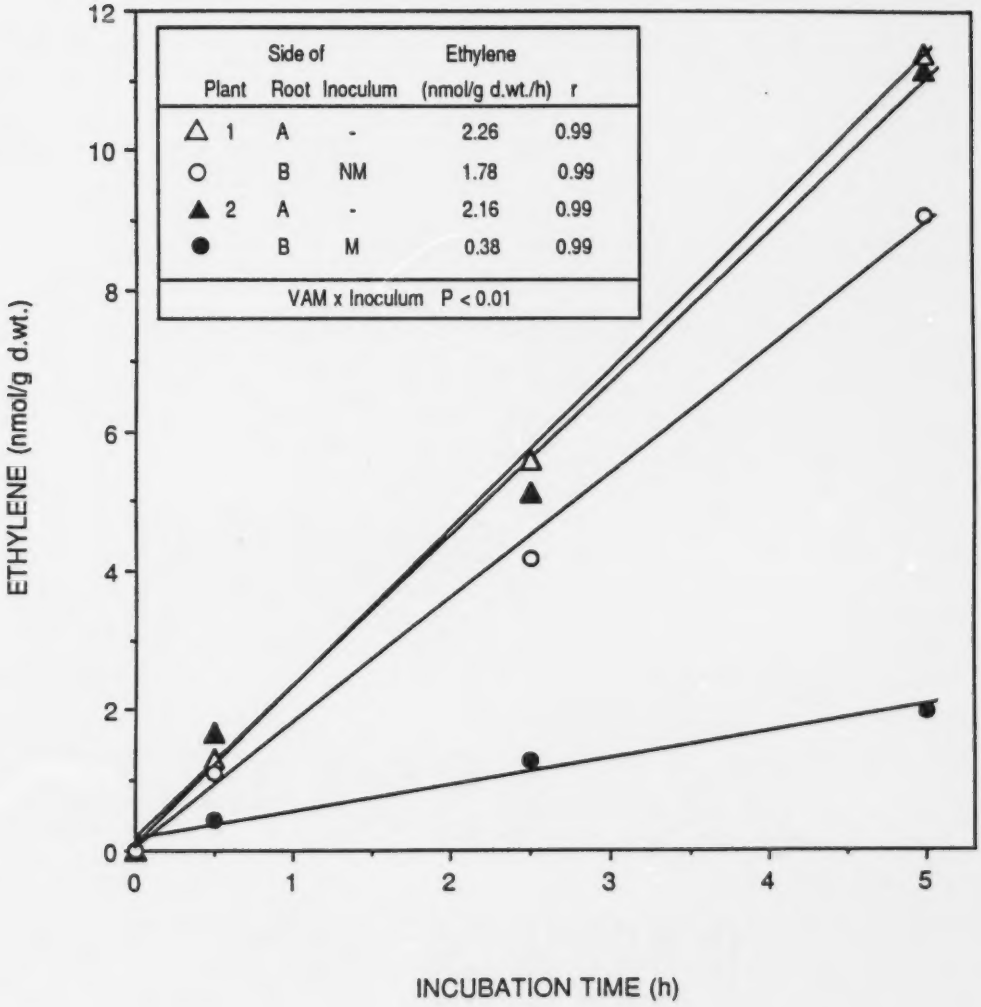

[P] mM
VAM
Ethylene (nmol/g d.wt./h)
r
0.5 - 0.96 0.99
+ 0.56 0.98
2.5 - 3.75 0.99
+ 0.94 0.99
[P] x VAM P < 0.01
ETHYLENE (nmol/g d.wt.)
INCUBATION TIME (h)
0
5
10
15
20
25
30
0
2
4
6
8

Figure V-4. Ethylene production (ACC_{ox} activity) by excised roots from 85-day-old potato plants that had one-half of their roots uninoculated (△, ▲) and the other half inoculated with NM (○) or VAM (●) (*G. fasciculatum*) clover inoculum. Roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 5.0 h. F-values for the main effects of VAM, Inoculum, $Time_{linear}$ and the interactions VAM x Inoculum, VAM x $Time_{linear}$, and Inoculum x $Time_{linear}$ were significant at the 0.05, 0.01, 0.01, 0.10, and 0.01 levels, respectively. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.


Side of
Plant Root Inoculum
Ethylene
(nmol/g d.wt./h) r
△ 1 A - 2.26 0.99
○ B NM 1.78 0.99
▲ 2 A - 2.16 0.99
● B M 0.38 0.99
VAM x Inoculum P < 0.01
ETHYLENE (nmol/g d.wt.)
INCUBATION TIME (h)
0
2
4
6
8
10
12
0
1
2
3
4
5

plant. Moreover, ACC_{ox} activity in roots exposed to NM inoculum was not significantly different than that from the uninoculated sides of NM or VAM plants.

When leachate from VAM roots was added to the incubation buffer of NM roots, ACC_{ox} activity and ethylene accumulation were inhibited by 75% relative to NM controls (Fig. 5 and inset). Furthermore, VAM-root leachate reduced the level of ACC_{ox} activity of NM roots to a level equivalent to that of VAM roots. No such inhibitory effect of VAM-root leachate was apparent when added to the VAM roots. Hence, it appeared that a potent water-soluble factor, unique to VAM roots, was responsible for the inhibition of ACC_{ox} activity. The results of these studies suggest that VAM fungi and increasing abiotic P-supply may influence the ethylene component of a plant's defense response in opposite ways, despite the fact that the net effect of either treatment was to enhance the plant's P nutrition.

In addition to the effects of abiotic P-supply and VAM infection on ACC_{ox} activity, other components of the defense response were examined in a separate study. Specifically, changes in phenolic production and the activities of POX and PPO in roots, key components of root defense responses to pathogens (5, 37), were quantified as a function of infection and level of P nutrition. These components may reflect the degree of resistance expressed by plants to VAM infection. From the data in Figure 6, it is evident that VAM colonization of roots in this study was less than that observed previously. The lower infection level was likely a consequence of less inoculum (50 g versus 100 g in the previous studies), earlier harvest dates, and a higher average concentration of P in roots (e.g., low P NM plants averaged 1.47 mg P g d.wt.$^{-1}$ versus 0.98 mg P g d.wt.$^{-1}$ in the previous study). Increasing the abiotic P-supply from 0.5 mM to 5.0 mM significantly reduced VAM colonization of roots (Fig. 6 inset); however, plants receiving 2.5 mM P showed the same level of infection as those receiving 0.5 mM P in this study. Moreover, fewer vesicles were present in the roots, and arbuscules and coiled hyphae were more prevalent as compared with that observed in the previous study. Although the level of infection increased from 21 to 42 DAP, infection levels remained constant between 28 and 35 DAP. Interestingly, this period coincided with tuberization of the VAM plants - tubers first appeared at 35 DAP. The reduced ability of VAM fungi to increase colonization during tuberization may be a consequence of a change in source to sink relationships favoring an increased flux of photoassimilates to tubers at the expense of roots and/or fungi, as has been suggested (22). On the other hand, our observations may reflect a more direct increased resistance response by the plant which is linked to developmentally induced changes in host compatibility.

Figure V-5. Ethylene production (ACC_{ox} activity) by excised roots from 83-day-old potato plants inoculated with NM (○, △) or VAM (●, ▲) (*G. fasciculatum*) clover inoculum and treated with 100 µL of incubation buffer (○, ●) or VAM root leachate (△, ▲). Roots were incubated in Mes (Tris) buffer (pH 6.5) containing 10 mM ACC at 23°C for 5.0 h. F-values for the main effects of VAM, Leachate, $Time_{linear}$ and the interaction of VAM x Leachate were all significant at the 0.01 level. Inset: Linear regression and correlation coefficients for ACC_{ox} activity of the various treatments.

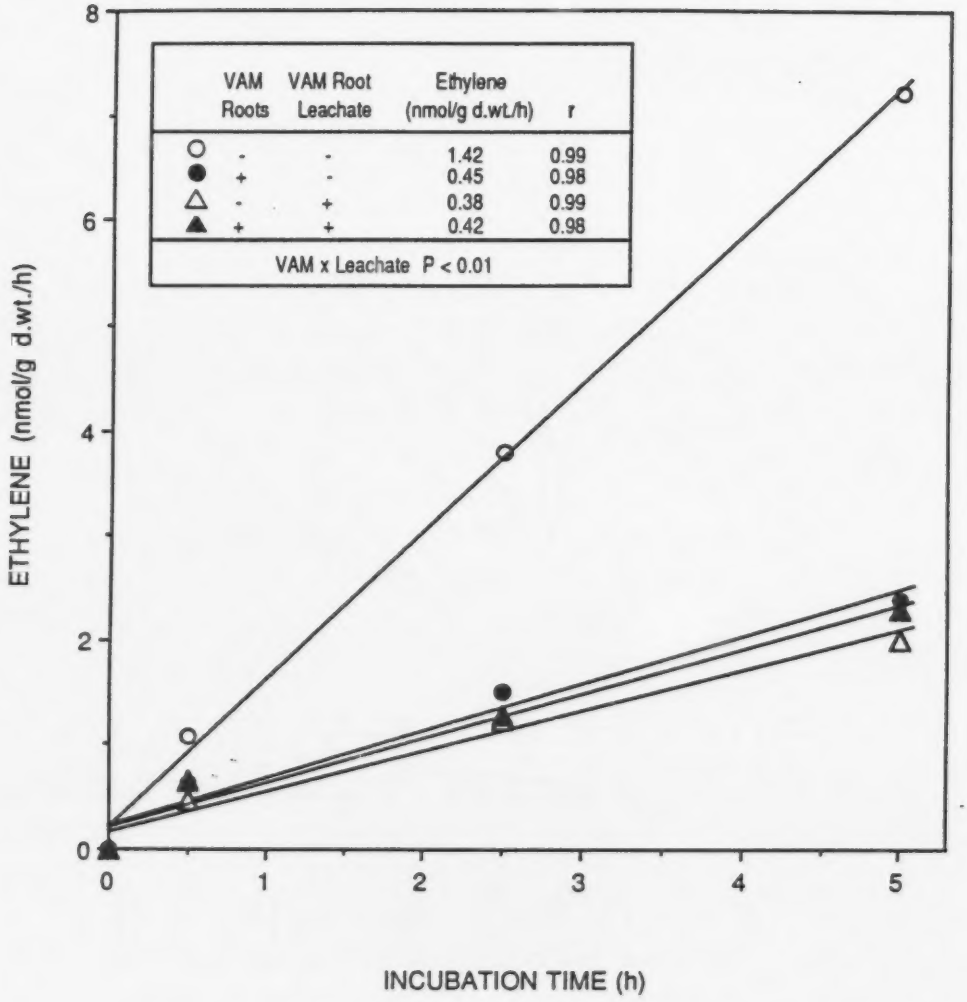
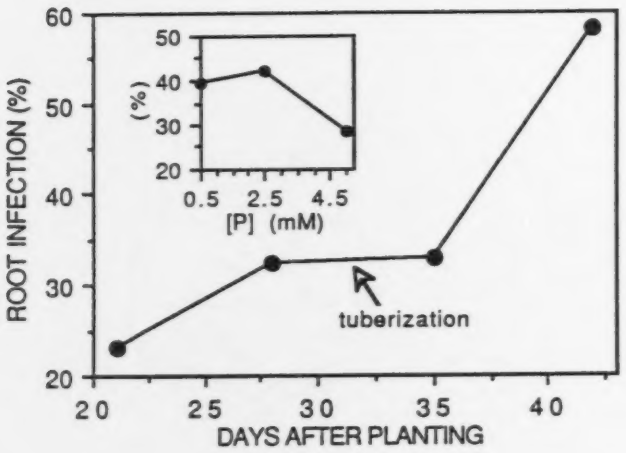

VAM Roots
VAM Root Leachate
Ethylene (nmol/g d.wt/h)
r
- - 1.42 0.99
+ - 0.45 0.98
- + 0.38 0.99
+ + 0.42 0.98
VAM x Leachate P < 0.01
ETHYLENE (nmol/g d.wt./h)
INCUBATION TIME (h)
0
2
4
6
8
0
1
2
3
4
5

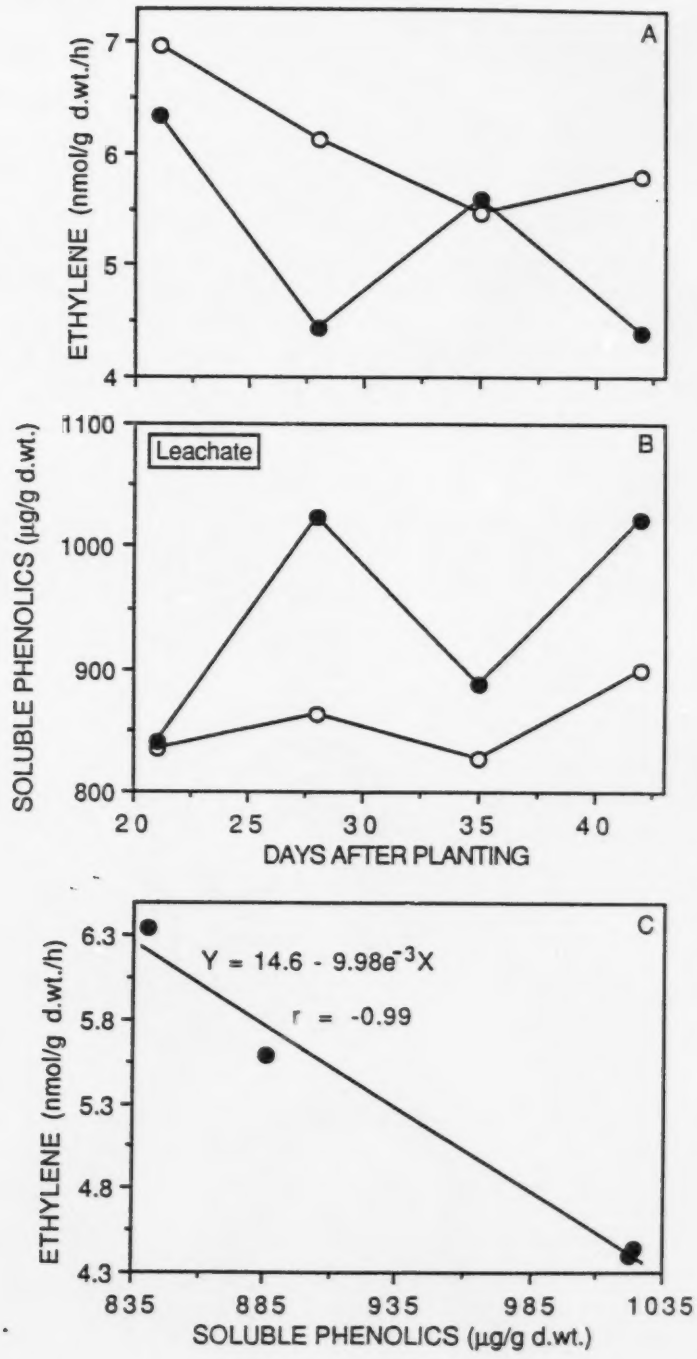
Figure V-6. Main effect of time on percent roots infected by *G. fasciculatum* for potato ants grown with 0.5, 2.5, or 5.0 mM P, and inoculated with VAM clover pot culture. values for the linear, quadratic, and deviation trends were significant at the 0.01, 0.05, and 0.05 levels, respectively. Inset: Main effect of increasing abiotic P supply on percent of roots infected. F-values for the linear and deviation trends were significant at the 0.01 and 0.05 levels, respectively.


ROOT INFECTION (%)
60
50
40
30
20
20
25
30
35
40
DAYS AFTER PLANTING
tuberization
(%)
50
40
30
20
0.5
2.5
4.5
[P] (mM)

With the exception of 35-day-old VAM roots, root ACC_{ox} activity decreased over the 42-day growth period (Fig. 7A), and the levels of activity in this study were higher than those observed previously (Fig. 3). The higher activities probably relate to higher root P concentrations in this study. Again, roots of plants supplied with higher levels of P had higher ACC_{ox} activities than those grown with low P. For every 1 mM increase in P supplied to plants during growth, root ACC_{ox} activity increased by 0.292 nmol g $d.wt.^{-1}$ h^{-1} (ACC_{ox} = 0.292[P] + 4.889, r = 0.99, $P < 0.05$). As in the previous study, VAM infection significantly decreased the ability of roots to produce ethylene from ACC, although the response was somewhat less. In this regard, the extent of inhibition of ACC_{ox} activity by VAM reflected the lower VAM infection levels in general, and especially the lapse in root colonization between 28 and 35 DAP. Unlike the earlier study, the [P] x VAM interaction on ACC_{ox} activity was not significant.

The effect of VAM infection on production of leachable phenolics from roots is illustrated in Figure 7B. Levels of leachable phenolics were not significantly affected by P nutrition or an interaction between VAM and [P]. Hence, Figure 7B shows leachable phenolics averaged over the three levels of P. The amount of leachable phenolics increased significantly with increasing DAP. Furthermore, VAM-infected roots leached significantly more phenolics than NM roots at each harvest date, although the differences between VAM and NM plants were lower at 35 DAP, reflecting the lack of increase in root infection during tuberization. For total extracted phenolics, the main effects of P and VAM infection were not significant; however, a highly significant VAM x $Time_{linear}$ interaction ($P < 0.01$) indicated that the increase in leached phenolics may be partly due to a 56% greater synthesis of phenolics by the VAM roots (4.3 versus 6.7 µg phenolics g $f.wt.^{-1}$ d^{-1} for NM and VAM roots, respectively). On a g f.wt. basis, the proportion of leached to extractable soluble phenolics was also significantly ($P < 0.01$) greater for VAM roots relative to NM roots (average of 29 and 34% for NM and VAM roots, respectively), which would also contribute to the higher levels of phenolics in leachates from VAM roots. A significant inverse correlation (r = -0.99) between the amount of leached phenolics and ACC_{ox} activity was apparent for VAM roots (Fig. 7C), while no such relationship existed for NM roots (data not shown; r = -0.19). These data suggest that the soluble component from VAM roots which inhibits ACC_{ox} activity (Fig. 5) may be of a phenolic nature.

Figure V-7. Time course of root ACC_{ox} activity (A) and soluble phenolic levels (B) in leachate of roots excised from NM (O) and VAM (*G. fasciculatum*) (●) infected potato plants. Roots were incubated in Mes (Tris) buffer at 25°C for 2.5 h. For ACC_{ox} activity, the buffer contained 10 mM ACC. F-values for the main effects of VAM and $Time_{linear}$ were significant at the 0.10 and 0.05 levels, respectively, for ACC_{ox} activity; for soluble phenolics, VAM, $Time_{linear}$, and $Time_{deviations}$ were significant at the 0.05, 0.10, and 0.05 levels, respectively. (C) Correlation between ACC_{ox} activity and leachate phenolic level for VAM roots. For NM roots, the correlation between ACC_{ox} activity and soluble phenolics was not significant ($r = -0.19$).


A
ETHYLENE (nmol/g d.wt./h)
7
6
5
4
B
Leachate
SOLUBLE PHENOLICS (µg/g d.wt.)
1100
1000
900
800
20
25
30
35
40
DAYS AFTER PLANTING
C
Y = 14.6 - 9.98e-3X
r = -0.99
ETHYLENE (nmol/g d.wt./h)
6.3
5.8
5.3
4.8
4.3
835
885
935
985
1035
SOLUBLE PHENOLICS (µg/g d.wt.)

Root PPO activity, which has been negatively correlated with susceptibility to pathogen invasion (5), increased to a maximum of 8.3 (ΔA_{475} g f.wt.$^{-1}$ min^{-1}) at 42 DAP in NM roots (Fig. 8). PPO activity on a f.wt. basis was not affected by VAM infection through most of the study, although VAM roots at 35 DAP had significantly higher activity ($LSD_{0.05}$ = 1.07 ΔA_{475} g f.wt.$^{-1}$ min^{-1}) than NM roots. The difference at 35 DAP may relate to the aforementioned lapse in VAM colonization (Fig. 6) due to tuberization between 28 and 35 DAP. The specific activity of PPO (data not shown) was significantly ($P < 0.01$) less (15%) in VAM roots due to an 18% higher rate of soluble protein accumulation in VAM roots, relative to that for NM roots (VAM x $Time_{linear}$ was significant at $P < 0.01$). Oddly, while increasing abiotic P-supply stimulated root ACC_{ox} activity and decreased VAM infection, suggesting a greater potential for a defense response, increased abiotic P-supply had a negative influence on PPO activity on a protein (data not shown) and f.wt. basis (Fig. 8 inset). Hence, our results provide no evidence supporting a role for PPO in the P-induced resistance response to VAM infection.

Peroxidase activity of roots, either in leachates (Fig. 9) or extracts (data not shown), increased over the harvest period. The specific activity of POX was 2.3-fold (NM) or 2.9-fold (VAM) greater in leachates relative to extracts. This enrichment of POX activity in leachate likely reflects the higher extracellular specific activity of POX seen in many plant tissues (21). On a f.wt. basis, VAM infection increased the POX activity of leachates but had no effect on POX activity in extracts from roots. Hence, infection significantly ($P < 0.01$) increased the proportion of leachable to extractable POX activity (27 and 34%, respectively, for NM and VAM roots). This result was similar to that established for phenolics and may reflect a role for some of these phenolics as substrates for extracellular POX (which is involved in lignin biosynthesis). In contrast, VAM infection did not appear to have a stimulatory influence on the specific activity of extracellular POX (Fig. 9B), since the specific activity of POX in VAM root extracts was less than that of NM root extracts (data not shown). These results are also similar to those established for PPO activity and probably reflect the greater protein content of roots in response to VAM infection. Since the proportion of leached POX to extracted POX activity on a mg protein basis was significantly increased by VAM infection (similar to that of POX on a f.wt. basis), it is likely that VAM fungi are somehow able to enhance POX activity in potato roots.

An influence of abiotic P-supply on POX activity was also observed, and this effect was similar to that for ACC_{ox} activity. The activity of POX in root leachates was 10% greater on a f.wt. basis (Fig. 9A inset) and 17% greater on a protein basis

Figure V-8. Main effect of time on polyphenol oxidase activity of roots from NM (○) or VAM (*G. fasciculatum*) infected (●) potato plants that were grown with 0.5 mM, 2.5 mM, or 5.0 mM P. F-values for the main effect of $Time_{linear}$ and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01 and 0.05 levels, respectively ($LSD_{0.05}$ =1.07). Inset: Main effect of increasing abiotic P supply on polyphenol oxidase activity of roots for NM or VAM plants. F-value for the linear trend was significant at the 0.01 level.

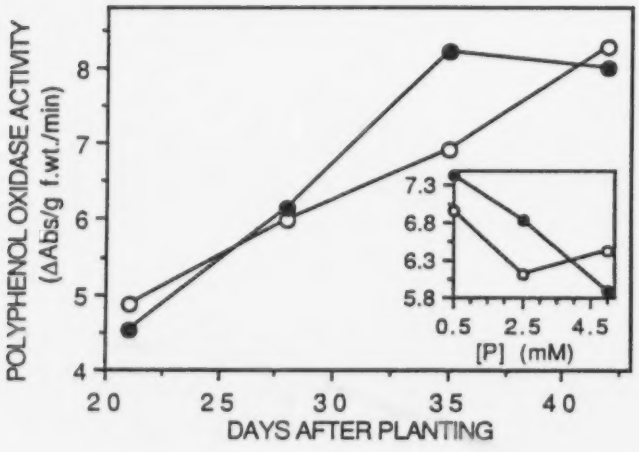
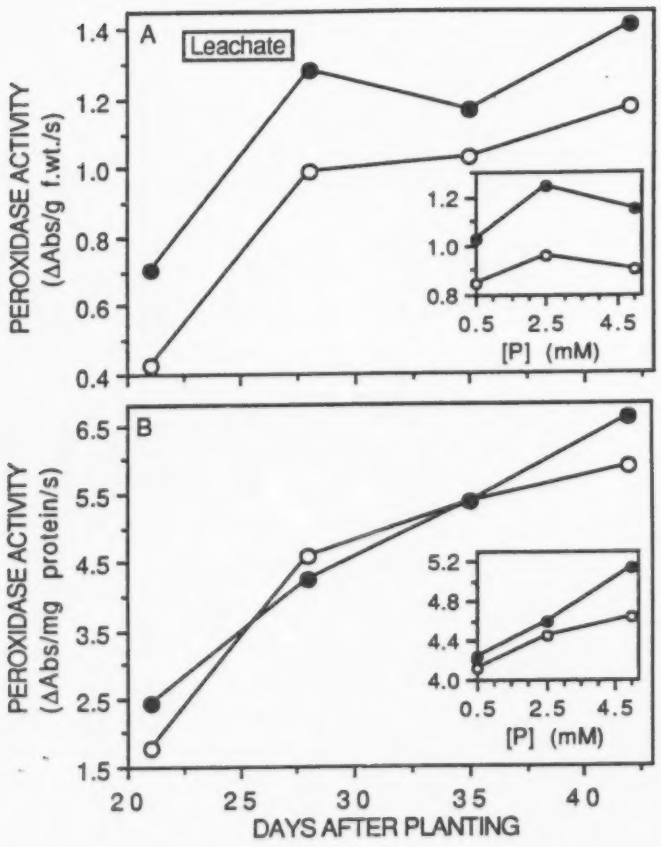

POLYPHENOL OXIDASE ACTIVITY
(ΔAbs/g f.wt./min)
8
7
6
5
4
20
25
30
35
40
DAYS AFTER PLANTING
7.3
6.8
6.3
5.8
0.5
2.5
4.5
[P] (mM)

Figure V-9. Time course of peroxidase activity in leachate from roots excised from potato plants inoculated with NM (○) or VAM (*G. fasciculatum*) (●) clover inoculum, and grown with 0.5 mM, 2.5 mM or 5.0 mM P. Roots were incubated in 50 mM Mes (Tris) buffer (pH 6.5) containing 25 mM NaCl at 25°C for 2.5 h. (A). POX on a g f.wt. basis, F-values for the main effects of VAM, $Time_{linear}$, $Time_{quadratic}$, $Time_{deviation}$, and $[P]_{deviation}$ (inset) were all significant at the 0.01 level. (B). POX specific activity, F-values for the main effects of $Time_{linear}$, $Time_{quadratic}$, $[P]_{linear}$ (inset) and the interaction of VAM x $Time_{quadratic}$ were significant at the 0.01, 0.01, 0.01, and 0.05 levels, respectively.


A
Leachate
PEROXIDASE ACTIVITY
(ΔAbs/g f.wt./s)
1.4
1.2
1.0
0.8
0.6
0.4
1.2
1.0
0.8
0.5
2.5
4.5
[P] (mM)
B
PEROXIDASE ACTIVITY
(ΔAbs/mg protein/s)
6.5
5.5
4.5
3.5
2.5
1.5
5.2
4.8
4.4
4.0
0.5
2.5
4.5
[P] (mM)
20
25
30
35
40
DAYS AFTER PLANTING

(Fig. 9B inset), at the highest relative to the lowest level of P. Activity of extracted POX was not significantly influenced by VAM infection as mentioned, but POX activity in extracts (f.wt. basis) was increased slightly by higher levels of abiotic P-supply ($P < 0.10$), similar to that for leachable POX activity. Electrophoretic studies of POX in our lab indicated that neither abiotic P-supply nor VAM infection altered the root isozymic patterns (unpublished data). This is not unusual since others have observed that roots, unlike other tissues, often constitutively express the plant's complete complement of POX isozymes (25).

Discussion

The negative effects of P deficiency on plant growth have long been documented, and there is an increasing body of knowledge on the physiological changes that occur in response to P deprivation (8, 18). These changes have been characterized as constituting a P starvation inducible metabolism (18). A detailed characterization of the influence of VAM infection on mineral physiology of potato during development of P deficiency showed that, while low P plants had significantly lower rates of root respiration, the activities of root microsomal ATPases and extracellular acid phosphatases were higher (Chapter III). Concomitant with these heightened enzyme activities were lower activities of ACC_{ox} (Fig. 3 inset) and extracellular POX (Fig. 9B inset), and higher levels of root infection by the VAM fungi (Fig. 6 inset). As abiotic P-supply increased, these trends reversed, suggesting that the plant's susceptibility to VAM infection is somehow linked to PSI metabolism of the roots. In this regard, choices between defense against infection by VAM fungi and adequate P nutrition appear to have been determined. Interestingly, the effects of increased nitrate supply on root ethylene biosynthesis and the extent of nodulation in legumes (26) appear analogous to the effects of P on ethylene biosynthesis and VAM infection.

In many host:parasite interactions, ethylene is usually associated with or induces various biochemical pathways considered integral to a plant defense response. For example, increased levels of ethylene produced by the plant upon infection can stimulate lignification of cell walls through increasing PAL and extracellular POX activities. Enzymes involved in the biosynthesis of phytoalexins (e.g., chalcone synthase) and other anti-microbial compounds (e.g., PPO), as well as chitinases are also enhanced in response to increases in ethylene during infection (7, 13, 37). Since increasing abiotic P-supply enhanced both ethylene production and extracellular POX activities in our studies, a similar defense response by potato roots may have been the basis for the observed P-induced resistance to VAM infection. Indeed, considering the aggressive

growth and invasive association that VAM fungi display within potato roots (Fig. 1), it seems likely that the plant would have some mechanism to limit invasion if abiotic P-supply was not limiting to plant growth.

Interestingly, studies by others have shown that VAM infection initially stimulates a high level of cell wall-bound POX (33) and chitinase (34) activities which, after about 20 DAP, decline to levels below that of controls. In those studies, plants were grown with only one level of abiotic P-supply (0.06 mM), and although tissue P concentrations were not reported, they would likely decline over time under such conditions. Hence, the initial stimulation observed in chitinase and POX activities in those studies may have reflected the plant's defense response to infection. With further plant growth and declining tissue P concentration, a shift away from defense activities by plant roots to PSI metabolism and, therefore, to an increased compatibility to the VAM infection would be expected. Since these resistance response enzymes are inducible by ethylene, their lower activities may have been in response to a VAM-induced suppression of ACC_{ox} activity, similar to that characterized in our studies.

Impairment of ACC_{ox} activity has been observed in other host:parasite interactions, such as during infection of grapefruit by *Penicillium digitatum* (1). Related studies have shown that inhibition of either ACC synthase or ACC_{ox} activity can result in redirection of SAM into the polyamine biosynthetic pathway and, as a consequence, result in further reductions of ACC_{ox} activity (14). Increased barley leaf polyamine levels implicate a parasite-induced enhancement of polyamine biosynthesis in the 'green island' (10) effect and a coincident decrease in ethylene biosynthesis by leaves. Similarly, VAM infection has been shown to increase the level of intermediates (e.g., arginine) and activity of enzymes involved in polyamine biosynthesis (11). A potential role for polyamines in the VAM-induced decrease in ethylene production of potato roots merits investigation.

The localized inhibition of ACC_{ox} activity by VAM infection (Fig. 4 inset), inhibition of ACC_{ox} activity by VAM root leachates (Fig. 5 inset), and an inverse correlation (Fig. 7C) between ACC_{ox} activity and total soluble phenolics from VAM root leachates, suggest a role for phenolic metabolism in the mechanism by which VAM fungi are able to suppress the plant's defense response. An influence of VAM fungi on phenolic metabolism was suggested by the higher level of phenolics in root leachate (Fig. 7B) and in root extracts by others (24). An important function for specific phenolics produced by roots, such as ferulic acid or cinnamyl alcohol, is as a substrate for extracellular POX in the lignin biosynthetic pathway. Lignin acts to strengthen cell walls, thereby providing a physical barrier to microbial invasion. While not examined in our studies,

observations by others suggest that roots show cell wall apposition to VAM hyphae; however, lignification is not enhanced at infection sites or even later after the infection has been well established (9, 16). Interestingly, ferulic acid and its conjugate 1-O-feruloyl-ß-D-glucose have been reported to be potent inhibitors of ACC_{ox} activity (30). If such phenolic compounds are involved in the observed inhibition of ACC_{ox} activity by VAM infection, a role for a conjugate of ferulic acid seems more likely, since ferulic acid can inhibit plant P uptake and VAM fungal growth (23, 36). It is worth noting that some fungi are capable of metabolizing plant phenolic compounds or producing phenolics themselves (31); however, the capabilities of VAM fungi in this regard have not been investigated.

Apart from the P-induced decrease in VAM infection of roots, it appeared that the host plant was exerting a certain degree of developmentally linked control over fungal growth (Figs. 6, 7 and 8). The lapse in the rate of infection from 28 to 35 DAP coincided with tuberization and was very similar to that observed during flowering of chrysanthemum (22). In the latter case, the initiation of flower buds coincided with a transient reduction in VAM infection. It was found that bud initiation correlated with decreased levels of root metabolites and exudates, and thus the inhibitory effect of flowering on VAM growth was attributed to a plant influence on fungal nutrition. While such an effect was not investigated in our study, VAM roots at 35 DAP had a level of ACC_{ox} activity (Fig. 7A) that was comparable to that of NM roots. Furthermore, the low ACC_{ox} activity of VAM roots during tuberization coincided with lower levels of soluble phenolics (Fig. 7B) and significantly higher root PPO activity (Fig. 8). Interestingly, some authors have suggested a role for endogenous ethylene production in tuber initiation (35), and this may have inadvertently triggered a defense response in the present study.

The results of our studies demonstrate that several components of a potato plant's defense response to microbial invasion are positively influenced by abiotic P-supply and suggest a role for the defense response in the observed P-induced decrease of VAM infection in roots. Under conditions of inadequate available P (plant P deficit), the potato plant shifts its priorities to P acquisition (PSI metabolism) and roots become more susceptible to VAM invasion. In this regard, ethylene production and phenolic metabolism in VAM roots appear to be of particular significance, although details of their interaction require further investigation. Elucidation of the metabolic basis for these changes in plant response will provide further insight to the mechanisms of compatibility of this symbiosis and will possibly be relevant to mechanisms of resistance in other plant:parasite interactions.

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Chapter VI

General Discussion and Conclusions

From the onset of this research, it was apparent that P, as the limiting factor for growth, not only dictated the extent of growth and development of potato, but also had a pleiotrophic influence on potato physiology, some unknown aspects of which defined the interactions between VAM fungi and host. Hence, it was evident that, to characterize physiological aspects of the interaction between these symbiotic fungi and potato, it would be necessary to, in some way, gauge how potato plants that were P deficient differed physiologically from those that were relatively P sufficient. As the actual site of the VAM symbiosis, roots were of particular interest. In this regard, it seemed quite probable that some changes to root physiology due to P deficiency reflected a lower growth potential, while others related to general mineral nutrient uptake, some specifically to P uptake, and others to factors regulating compatibility to the VAM symbiosis.

Results from each of the growth studies demonstrated a significantly lower rate of biomass accumulation and total leaf area expansion for plants grown with a low level of P nutrition (no-P or 0.5 mM P) in comparison to those with high-P (2.5 and/or 5.0 mM P). Diminished photosynthetic surface area for low-P plants resulted mainly from an inhibition of lateral shoot growth and leaf expansion. The influence of P nutrition on tuber growth varied between studies; however, tuber growth was generally inhibited by low P and the extent of this presumably depended upon the timing of tuber initiation in relation to the onset of P-deficiency stress. For plants harvested at 42 DAP (Chapter IV), P deficiency almost totally prevented tuber growth, possibly reflecting the importance P to both photosynthesis and cell division (Bieleski and Ferguson, 1983). In each study, restricted growth and development in general were primarily associated with a diminished P content and tissue-P concentration, although some reduction in uptake and assimilation of other mineral-nutrients could also be inferred. In this latter regard, however, low-P plants did not express any other mineral deficiency than P (mineral accumulation was in general, however, significantly less than for high-P plants), nor for that matter did they appear to be carbohydrate-starved (Chapter III). These results plus the observation that plant stunting, leaf darkening, and purpling on the undersides of leaves were evident only for low-P plants, are consistent with descriptions of P deficiency of potato and most other plants (Marschener, 1986).

The extent of growth inhibition as a result of P deficiency differed between plant organs. Root growth, which was one-tenth as responsive to P nutrition as shoot growth (Chapter II and IV), was also much less affected by P deficiency, as reported by Cogliatti and Clarkson (1983) for the potato cultivar Desiree. Maintaining root growth over shoot or tuber growth under conditions of inadequate mineral nutrition has an obvious adaptive value. Roots of P-deprived plants in the study by Cogliatti and Clarkson and in other studies (Lee, 1982; Goldstein et al., 1989) also demonstrated an enhanced ability to absorb P, and transported relatively less P to the shoot than plants with an uninterrupted supply of P. Roots of P-deficient plants also show changes to ion-selectivity that restrict absorption of other mineral nutrients (Clarkson et al., 1989; Lee, 1982; Rufty et al., 1990). Although the basis for such changes to mineral nutrient uptake and transport is not yet known, allosteric regulation, regulation through control of ion efflux to the xylem, and also turnover of ion-specific carrier proteins have been suggested to be involved (Drew and Saker, 1984; Glass, 1990; Lee, 1982).

In the present studies, kinetic analysis of mineral nutrient uptake by potato roots was not attempted; however, some indirect evidence suggests that P-deficiency stress altered the selectivity of roots for mineral nutrient uptake. Hence, the accumulation of minerals other than P was decreased and yet, low-P plants consistently accounted for a higher proportion of available P than high-P plants. A reduction in root dry mass and hence, absorptive surface area (Newman and Andrews, 1973), and also a lower root respiration rate could account for some of the difference in the mineral-status between low-P and high-P plants. The same argument, however, rules out an influence of P deficiency on root morphology as being responsible for a higher accounting of available P. In fact, no influence of P on morphology was obvious for root samples in general, nor during percentage infection determinations. On the other hand, root physiology was altered by P deficiency in a fashion that was consistent with the induction of a 'phosphate starvation rescue system' (Goldstein et al., 1989). In this regard, the activity of both extracellular (secreted) and total Apase of potato roots were inversely related to abiotic P-supply.

The enhanced activity of such phosphatases is considered to be an integral component of plant response to P deficiency and is generally associated with the increased absorptive capacity of roots for P (Duff et al., 1991; Goldstein et al., 1989; Lee, 1988). Possibly related to these observations, the specific activity of vanadate-sensitive ATPases in root microsomes was also enhanced by P deficiency (Chapter III). Higher ATPase activity may represent an attempt by P-deficient cells to increase the proton motive force across their plasma membranes. Such activity could stimulate

Apase activity and is also required for uptake of phosphate. Although acidification of the rhizosphere by such activity was not measured for potato roots, enhanced ATPase activity could also result in more rapid dissociation of P-containing compounds and thus, promote phosphate uptake (Glass, 1990; Leonard, 1985; Marschner, 1986). While of a preliminary nature in themselves, these results characterize some physiological changes to roots of potato in response to P deficiency, and in the context of what is known from other studies, it appeared that these changes had been induced to improve root acquisition and utilization of P. The actual contribution of such alterations to efficiency of P uptake, however, is difficult to estimate, as equivalent P-deficient plants without such physiological changes were not available for this study. In contrast, the effect of the VAM symbiosis on plant P acquisition was measurable. Moreover, as indicated by the inverse relation between P nutrition and level of root infection, plants responded to P deficiency by increasing their compatibility to VAM fungi and thus, by virtue of producing mycorrhizae, became more efficient at P acquisition.

The morphology of potato roots infected by *Glomus fasciculatum* was characterized using light and electron microscopy (Chapters II and V). These observations demonstrated fungal structures typically associated with a VAM symbiosis (Holley and Peterson, 1979), and moreover, depicted these fungi as being strongly invasive in potato roots. In some cases, a vesicle or an arbuscule almost completely occupied the cell lumen. No contaminating microbes were observed in roots of NM or VAM plants.

Each of the four *Glomus* species used in these studies produced an extensive root infection of the potato cultivar Russet Burbank, and promoted growth, development and mineral accumulation. Comparison of species for their ability to colonize roots showed that species ranked *G. intraradices* > *G. mosseae* > *G. dimorphicum* and, as observed by others, the more rapidly a species colonized roots, the greater the effect on plant growth and development (Abbott and Robson, 1982). Rapid fungal growth is likely to be more effective at promoting plant growth, as it is necessary to establish both an internal fungus - host interface for nutrient exchange and also external hyphae for P acquisition (Schwab et al., 1991). Regardless of species, the level of root infection and plant response to the VAM symbiosis depended upon the abiotic P-supply to plants (Koide, 1991).

Root colonization progressed slowly for high-P plants, indicating a lower host-compatibility compared to low-P plants (Chapter V; Koide and Schreiner, 1992); moreover, a growth response for high-P plants to the VAM symbiosis was only marginally evident. In some studies (for example, Chapter II), a significant increase in P accumulation in response to the VAM infection was demonstrated even though the

asociated growth response was small. While an improved P-status for high-P VAM plants might indicate an enhanced potential for tuber production later, these results also reflect the cost, in terms of P utilization efficiency, of the VAM symbiosis to the plant (Koide, 1991). This cost is also reflected in the lower ULR of VAM plants compared with NM plants. It is of interest to note, however, that for plants grown with the same abiotic P-supply, no reduction in growth for VAM plants *versus* NM plants was evident in any of these studies.

Although tissue-concentration and/or accumulation of various mineral nutrients were increased in response to the VAM symbiosis, alleviation of P deficiency was clearly the primary benefit to plants. Higher plant tissue-concentration and accumulation of P in response to VAM species was the only consistently demonstrated effect of VAM fungi on plant mineral nutrition. VAM plants also accounted for a higher proportion of available P than NM plants, reflecting the contribution of external hyphae to the total absorptive surface area of mycorrhizae (Bolan, 1991). In this regard, the ratio of P-accumulated to P-available for VAM plants increased concurrently with root infection level and, similar to the level of root infection, was decreased by high abiotic-P supply.

Physiological attributes of VAM roots were compared with those of NM roots. Enzyme activity previously characterized as above on NM plants, i.e. typically associated with P metabolism, and also some typical of the plant defense response (Ward, 1986) were especially of interest. Complicating any such analysis of mycorrhizae, however, is the initimate, intraradical association between participants. Although some enzyme analysis of intraradical structures was accomplished in one study (Capaccio and Callow, 1982), no study to date has accomplished the separate physiological characterization of plant cells interacting with intraradical hyphae. Hence, any comparison of NM and VAM roots should acknowlege the confounding effects of the presence of fungal metabolism or constituents. It is of interest to note, however, that during the electrophoretic characterization of extracted proteins from potato roots, there was little evidence for extraction of significant amounts of any fungal protein or Apase activity on any of four harvest dates (Chapter IV). This finding may reflect on the fineness and quantity of the intraradical hyphae and also the largely lipid content of intraradical structures; it also indicates that the following results on enzymes are less confounded by the presence of fungal protein than might at first be expected.

Inducible Apase activity is implicated in the plant P starvation rescue system (as above) and also in nutrient exchange, as a component necessary for the operation of a triose-P translocater (Schwab et al., 1991). The specific activity of Apases in either microsomal or soluble fractions extracted from roots was decreased in response to VAM

infection, apparently reflecting the improved plant P nutrition with the VAM symbiosis (Chapter II and IV). Isozyme analysis of Apase activity demonstrated no change in response to the VAM symbiosis. It is of interest to note that the specific activity of microsomal ATPases, which has also been characterized as important both for P uptake and also for symbiotic nutrient exchange was not altered by the VAM symbiosis. These latter results appear to be in contrast to the cytochemical characterization of ATPase activity of periarbuscular membranes by Gianinazzi-Pearson et al. (1991). The findings on potato indicate that if enhanced activity of these enzymes at the interface between plant cell and fungus is important to nutrient exchange, it is not likely through specifically enhanced synthesis of these enzymes.

It is still possible, however, that high localized activity of either plasma membrane ATPases or Apases in the interfacial matrix occurs, possibly stimulated by hormones suggest as auxin, or by turgor changes for example (Gianinazzi-Pearson et al., 1991). Also, high P nutrition could interfere with nutrient exchange between symbiont and host by decreasing the level of Apase activity in the matrix. Perhaps of more significance, the VAM symbiosis promoted a large increase in protein and (apparently) plasma membrane content of cells, probably by arbuscular invasion (Cox and Tinker, 1976). The resulting enhanced root content of both soluble and microsomal Apases, and also microsomal ATPases substantialy increased such activities on a fresh weight basis. Such a result appears to demonstrate a heightened root potential for nutrient exchange with intraradical hyphae.

A role for plant resistance responses in host compatibility to the VAM fungi appears plausible by the demonstration of an influence of P nutrition on root capacity for ethylene generation (Drew et al., 1989). In this regard, ACC_{ox} was shown to be much lower for roots of P-deficient potato plants than for those with relatively sufficient P. Perhaps the most interesting finding from the studies herein was the demonstration that the VAM symbiosis significantly decreased ethylene production and ACC_{ox} activity in particular, even while improving the P nutrition of plants. Diminished ACC_{ox} activity in VAM roots was associated with a lower polyphenoloxidase activity in root extracts, while peroxidase activity was unchanged. These results characterize a low resistance response of low-P plants to VAM infection. High-P nutrition stimulated an increase in ACC_{ox} and peroxidase activity, and was associated with a diminished level of root infection. These finding merit further investigation.

In summary, the effects of P deficiency on the potato cultivar Russet Burbank, were shown to be pleiotrophic and were characterized in terms of plant growth, development and mineral physiology. Plants demonstrated physiological changes in response to P

deficiency that improve root P acquisition, with receptivity to the VAM symbiosis being perhaps the most beneficial. Changes that occurred in roots throughout the development of the mycorrhizae were complex, but appeared to reflect improved P nutrition, an expansion of cell capacity for nutrient exchange and an increase in host compatibility. Changes to compatibility appeared to at least partially involve the defense response of plants. The VAM symbiosis provides a model system for investigating the effects of environmental factors, such as P, on plant resistance mechanisms that may have implications for parasite:host interactions, in general.

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